university of groningen

Cardiorenal biomarkers and therapeutic interventions in acute heart failure

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de rector prof. dr. E. Sterken
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 26 augustus 2019 om 16:15 uur

Yuya Matsue

geboren op 23 januari 1980

te Toyama, Japan

Promotores

Prof. dr. A.A. Voors

Prof. dr. P. van der Meer

Co-promotor

Dr. K. Damman

Beoordelingscommissie

Prof. dr. R. A. de Boer

Prof. dr. G. J. Navis

Prof. dr. W. Mullens

Paranimfen

Dr. Tom Hendriks

Dr. M. Abdullah Said

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Chapter 1

Introduction

Acute heart failure (AHF) is one of the leading causes of hospitalization and death worldwide. More than one million people in the United States and Europe, and more than 200,000 people in Japan are hospitalized each year. AHF-associated mortality is unsatisfactory high, and there is no specific treatment that has been shown to improve prognosis. Although the pathophysiological background of AHF is multifactorial, renal dysfunction is among one of the most common and powerful prognostic factors. The overwhelming amount of data related to the prognostic importance of renal function and worsening renal function in patients with heart failure was summarized in a recent meta-analysis. In this meta-analysis, it was reported that 49% of patients with heart failure had concomitant renal dysfunction, which is typically defined as an estimated glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2. Worsening renal function was present in 23% of patients. Moderate renal dysfunction (hazard ratio 1.59, 95% confidence interval [CI] 1.49-1.69), severe renal function (hazard ratio 2.17, 95% CI 1.95-2.40), and worsening renal function (hazard ratio 1.95, 95% CI 1.45-2.62) were independently associated with mortality. These findings imply the prognostic importance of renal function in patients with heart failure.

Despite the presence of such detailed prognostic information, surprisingly little is known regarding the pathophysiological mechanism and treatment of unfavorable heart-kidney interplay in patients with AHF. Renal dysfunction in patients with heart failure has been believed to be associated more with cardiac output or ejection fraction than congestion. Many recent studies, however, imply there is no or very weak, if any, association between cardiac output and renal function, and venous congestion might be a more prominent driver of renal dysfunction in patients with heart failure. Some studies, which have tested an association between changes in serum creatinine and prognosis in patients with AHF, showed that an increase in creatinine does not always lead to a worse prognosis, and should be interpreted in the context of the clinical course. Moreover, many studies have shown that not only glomerular but also tubular function is impaired and independently contribute to the unfavorable association between renal dysfunction in general and worse outcomes. Nevertheless, the terms “renal function” and “(estimated) GFR” are often used interchangeably. It is well known that creatinine has important limitations, even as a biomarker of glomerular function. Creatinine is influenced by some non-renal factors including muscle mass, diet, and ethnicity, and there is a non-negligible discrepancy between GFR estimated from serum creatinine levels and true GFR in patients with heart failure. Also, because creatinine does not rise until GFR decreases by 50% and does not show dynamic changes with GFR, it is not an ideal biomarker to monitor (acute) changes in glomerular function. One of the factors that hampers further intensive clinical research aimed at revealing this complex association is a lack of renal biomarkers to better encompass and reflect the underlying pathophysiological background of renal function. Because the absolute value and serial changes of serum creatinine or eGFR do not contain any information on pathophysiological background, it is not plausible to design the study using creatinine as an outcome measure alone. Furthermore, several randomized clinical trials that tested drug targeting in AHF patients with concomitant renal dysfunction showed neutral results in terms of prognosis, and many questions remain unanswered. It is undisputable that there is a need for novel renal biomarkers to better understand this multifactorial and complex, but clinically relevant heart-kidney interaction in patients with AHF.

The kidney is not the only organ impaired in patients with AHF, and several recent studies showed that many other

organs, including liver, bone marrow, brain, intestine, and lung are also implicated in unfavorable organ cross-talk. Not surprisingly, outcome is more negatively impacted when increasingly more organs are impaired. Congestion is one of main players in the pathophysiological background of AHF, and is also one of the main drivers of organ dysfunction in patients with AHF. For instance, venous or systemic congestion can lead to increased ventricular wall stress, myocardial stretch, and subsequent myocardial necrosis, which can be detected using cardiac troponin. Hepatic dysfunction is associated with heart failure due to increased venous pressure and reduced hepatic blood flow leading to elevated cholestatic enzymes, transaminases, and bilirubin. Of note, a biomarker sub-study, RELAX-AHF, showed that the prognosis in organ damage over time in AHF together with the degree of each organ dysfunction can affect future prognosis. According to these findings, it could be hypothesized that early decongestion might prevent further damage to organs. Indeed, the concept of early treatment in patients with AHF has been launched in the latest Heart Failure Guidelines of the European Society of Cardiology in 2016. However, recently performed clinical trials have focused more on the intervention itself and less on the time to intervention. Based on this review of the current literature and the gaps identified therein, testing the hypothesis that providing effective decongestion treatment at appropriate times as a key to improving outcomes of AHF patients is of particular interest from a clinical and scientific perspective.

Aims of this thesis

Although many novel renal biomarkers have been tested and shown to be associated with prognosis in patients with heart failure, very few of them were associated with pathophysiological background of cardio-renal interaction in heart failure. In the first part of this thesis, I aim to evaluate a novel renal biomarker and pre-existing metric of renal function to identify its role and explore the possibility that they might provide us with pathophysiological and prognostic information which cannot be achieved by pre-existing biomarkers. Chapter 2 examines the position of the novel cardio-renal biomarker, proenkephalin, in patients with heart failure. Chapter 3 defines the normal range of the blood urea nitrogen-creatinine ratio using the general population, and identifies the prognostic implication of the blood urea nitrogen-to-creatinine ratio in patients with acute heart failure.

In the next part of this thesis, I test the hypothesis that early treatment with a novel diuretic could be an option for patients with acute heart failure with concomitant renal dysfunction. Although renal dysfunction is one of the comorbidities which relate to poor treatment response and outcomes in patients with AHF, specific treatment for this high-risk subgroup has yet to be developed. Chapter 4 describes the rationale and design and Chapter 5 summarizes the results of the clinical utilities of early treatment with vasopressin-2 receptor antagonists in patients with AHF with concomitant renal dysfunction in a randomized clinical trial (AQUAMARINE study). Chapter 6 investigates the effects of an early adjunctive therapy with tolvaptan on the diuretic response in AHF patients with renal dysfunction.

In Chapter 7, we aim to study the effects of early loop diuretic therapy on short-term prognosis in patients with AHF. Although the idea “early treatment provides better prognosis” has been around for a long time in AHF, no study adequately and specifically tested this hypothesis.

Finally, findings and future perspectives are discussed in summary and future perspectives.

Chapter 2

Clinical Correlates and Prognostic Value of Pro-Enkephalin in Acute and Chronic Heart Failure

Yuya Matsue
Jozine M. ter Maaten
Joachim Struck
Marco Metra
Christopher M. O'Connor
Piotr Ponikowski
John R. Teerlink
Gad Cotter
Beth Davison
John G. Cleland
Michael M. Givertz
Daniel M. Bloomfield
Howard C. Dittrich
Dirk J. van Veldhuisen
Peter van der Meer
Kevin Damman
Adriaan A. Voors

J Card Fail 2017;23:231-239

Abstract

Background

Proenkephalin (pro-ENK) has emerged as a novel biomarker associated with both renal function and cardiac function. However, its clinical and prognostic value have not been well evaluated in symptomatic heart failure patients.

Methods and Results

The association between pro-ENK and markers of renal function was evaluated in 95 chronic heart failure patients who underwent renal hemodynamic measurements including renal blood flow (RBF) and glomerular filtration rate (GFR) using131I-Hippuran and 125I-Iothalamate clearances, respectively. The association between pro-ENK and clinical outcome in acute heart failure was assessed in another 1589 patients. Pro-ENK was strongly correlated with both RBF ($P<0.001$) and GFR ($P<0.001$), but not with renal tubular markers. In the acute heart failure cohort, pro-ENK was a predictor of death through 180 days, heart failure rehospitalization through 60 days, and death or cardiovascular or renal rehospitalization through day 60 in univariable analyses, but its predictive value was lost in a multivariable model, when other renal markers were entered in the model.

Conclusions

In patients with chronic and acute heart failure, pro-ENK is strongly associated with glomerular function, but not with tubular damage. Pro-ENK provides limited prognostic information in acute heart failure patients on top of established renal markers.

Introduction

Renal dysfunction is frequently observed in heart failure patients[1], and both baseline renal function and worsening of renal function accompanying inadequate decongestion during hospitalization is associated with prolonged hospitalization, rehospitalization, and death[2, 3].

Enkephalins including pro-enkephalin (pro-ENK) are small endogenous opioid peptides encoded by the proenkephalin gene, and have been shown to be implicated in neurotransmission, autocrine and paracrine function, and cardiac function. Most of the early studies have focused on its role in neuronal tissues, but it is also suggested to be produced and act in non-neural tissues including heart and kidney[4]. Due to the instability of enkephalins, a stable fragment of their precursor, termed pro-ENK, has been devised as stable and reliable surrogate plasma marker[5]. In patients with acute kidney injury after cardiac surgery, pro-ENK was shown to rapidly increase[6]. In acute myocardial infarction, increased pro-ENK was associated with renal dysfunction and predicted major cardiac events[7]. These results suggest a potential of pro-ENK as a novel cardiorenal biomarker, although its role in chronic and acute heart failure has not been established. Here, we evaluate the association between pro-ENK and indices of glomerular and tubular function and clinical outcome in patients with acute and chronic heart failure.

Methods

This study was performed in two populations. First, a cardiorenal mechanistic cohort was used to investigate the association between pro-ENK and renal function including hemodynamic parameters which were measured by radioactive tracers in stable chronic heart failure patients[8, 9]. Second, the PROTECT (Placebo-controlled Randomized study of the selective A1 adenosine receptor antagonist rolofylline for the patients hospitalized with acute heart failure and volume Overload to assess Treatment Effect on Congestion and renal functTion) study cohort (acute heart failure cohort) was used to study the association between pro-ENK and prognosis in patients with acute heart failure[10]. Measurement of pro-ENK was performed using a sandwich immunoassay with antibodies against the proenkephalin A 119-159 peptide by Sphingotec inc.[5, 7]. The lower detection limit was 5.5 pmol/L. Intra- and inter-assay coefficients of variation were 6.4 and 9.5% at 50 pmol/L, and 4.0 and 6.5% at 150 pmol/L, respectively. The normal value of pro-ENK was measured in a general population, and determined as 46.6 $\pm$14.1 pmol/L and median value of 45 (range: 9-518) pmol/L[11]. The 99th percentile upper reference limit of pro-ENK in healthy subjects was 80 pmol/L[11].

Renal mechanistic cohort (chronic heart failure)

Patient selection and measurement procedure of renal hemodynamic parameters have been described elsewhere[8, 9]. In brief, 120 ambulatory heart failure patients with left ventricular ejection fraction (LVEF) <45% on stable doses of ACE inhibitor or ARB for at least one month were included at University Medical Centre Groningen. All patients who consented to participate underwent GFR and effective renal plasma flow measurement using ^{125}I-Iothalamate and ^{131}I-Hippuran. Renal blood flow (RBF) was calculated as effective renal plasma flow/1-haematocrit. GFR and

RBF were expressed per body surface area. pro-ENK values were measured in 95 available plasma samples. Serum cystatin C levels were measured by nephelometry. Urinary tubular markers including neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-beta-D-glucosaminide for N-acetyl-β-D-glucosaminidase (NAG), and Kidney Injury Molecule 1 (KIM-1) were also determined by ELISA as previously described[9].

Acute heart failure cohort

We also measured pro-ENK in the PROTECT study cohort. The details of the design, results, and conclusions of this study have already been published[10, 12, 13]. In brief, 2,033 acute heart failure patients with renal function impairment (estimated creatinine clearance between 20 to 80 mL/min with Cockcroft–Gault formula) were included and randomized to rolofylline or placebo. The protocol of the PROTECT study was approved by the ethics committee at each participating center, and written informed consent was obtained from all participants. We measured pro-ENK in 1,589 patients at baseline (day 1) 1,465 patients at day 2, and 1,200 patients at day 7 as samples were available. The following biomarkers were also evaluated at baseline; albumin, blood urea nitrogen (BUN), creatinine, glucose, hemoglobin, potassium, sodium, total cholesterol, triglycerides, uric acid and white blood cell count were measured by ICON Laboratories, Farmingdale, New York. N-Terminal pro Brain Natriuretic Peptide (NT-proBNP) was determined by screening using commercial assays available at study sites. NGAL and C-reactive protein were measured in available frozen plasma samples by Alere Inc., San Diego, CA, USA. NGAL was measured using sandwich enzyme-linked immunosorbent assays (ELISA) on a microtiter plate; C-reactive protein was measured using a competitive ELISA on a Luminex platform.

We also evaluated the association between worsening renal function (WRF), pre-defined in PROTECT as a creatinine increase of $\geq$0.3 mg/dL from baseline (day 1) value or initiation of hemofiltration or dialysis at any time between day 1 to day 4.

The prognostic value of pro-ENK was evaluated with 1,589 AHF patients with available Pro-ENK value at baseline using three endpoints: all-cause mortality within 180 days, heart failure rehospitalization through 60 days, and death or cardiovascular or renal rehospitalization through day 60 days[14].

Statistical analysis

In both cohorts, data are expressed as mean and standard deviation for normally distributed variables, and as median with interquartile range for non-normally distributed data. Categorical data are expressed as numbers and percentages. The relationship between baseline characteristics and tertiles of pro-ENK were compared by using one-way analysis of variance test, Kruskal-Wallis test, or chi-squared tests where appropriate. A post-hoc test for pairwise comparison was performed with Bonferrroni correction. When necessary, variables were transformed for further analyzes. Stepwise multiple linear regression analysis was performed using backward elimination with a P value <0.10 as the criterion for retention after including all variables with P value <0.10 in univariate analysis to identify factors independently associate to pro-ENK levels.

In the acute heart failure cohort, univariate logistic regression was performed to evaluate predictability of pro-ENK for WRF. If pro-ENK was significant in univariate logistic regression, multivariable logistic regression was performed to adjust for baseline creatinine levels to evaluate additive predictability for WRF. The longitudinal trajectory of

pro-ENK over time (day 1, day 2 and day 7) was assessed by using linear mixed effect models to account for within-individual correlation of repeatedly measured values of pro-ENK. For this analysis, we excluded patients who died within 7 days. Identification of subjects was included as random effects, and time was modeled linearly. We used age, previous heart failure hospitalization, peripheral edema, systolic blood pressure, serum sodium, log blood urea nitrogen, log creatinine, and albumin as fixed effects as these were suggested as factors of prognostic predictive value in this cohort[15]. For prognostic analysis, we adjusted log pro-ENK by a model that was previously defined for this cohort, including age, previous heart failure hospitalization, peripheral edema, systolic blood pressure, sodium, log blood urea nitrogen, log creatinine and albumin.[15]. In this cohort, predictability of this model was confirmed to be similar to more complex models for outcome of all-cause mortality within 180 days, death or rehospitalization for any reason within 30 days, and cardiovascular or renal rehospitalization within 30 days. We evaluated prognostic predict ability of pro-ENKN in three multivariable Cox models: adjusted for age and gender (Model 1), adjusted for age, gender, creatinine, and BUN (Model 2), and adjusted for the clinical model (Model 3). A two-tailed P value < 0.05 was considered statistically significant. Statistical analyses were performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). ISBN 3-900051-07-0, URL http://www.R-project.org.

Results

Renal mechanistic cohort

Patient characteristics

Baseline characteristics are shown in Table 1. The mean age was 60±12 years, 75 patients (79%) were male, and mean LVEF was 29±10%. The median value of pro-ENK was 62.2 (IQR: 48.5 –92.5) pmol/L (Figure1), and 28 (29.5%) patients had pro-ENK levels above 99th percentile upper reference limit of pro-ENK in healthy subjects. Higher pro-ENK tertiles were associated with higher age, females, lower blood pressure, higher NYHA class, greater diuretics use and higher plasma NT-proBNP levels (all *P*<0.05).

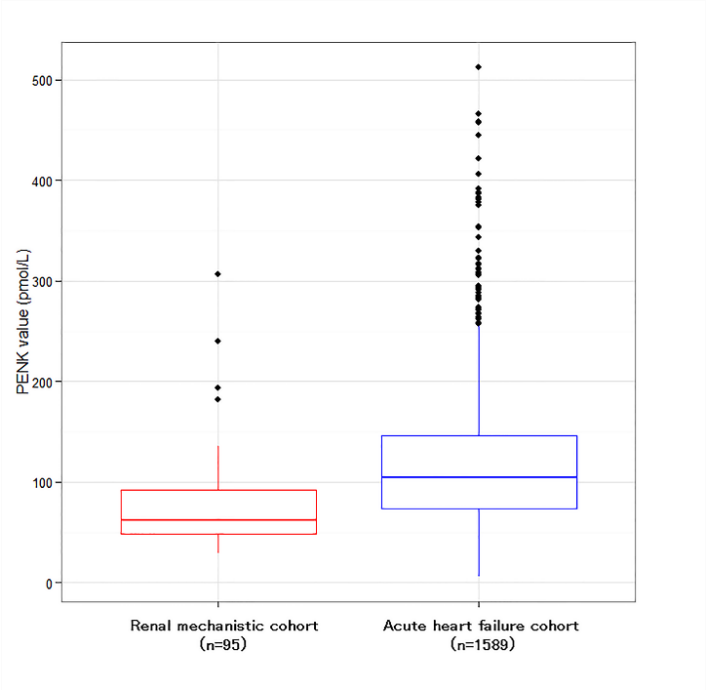

Figure 1. Baseline pro-ENK values in renal mechanistic cohort and acute heart failure cohort
The box represent interquartile ranges, the horizontal line in each box represents the median, and the whiskers show the 10-90 percentile range.

Correlation between renal markers and pro-ENK

Supplemental Table 1 shows the result of univariate linear regression analysis between log pro-ENK, renal markers and renal hemodynamic parameters. pro-ENK values were strongly and significantly associated with creatinine, BUN, Urinary Albumin Excretion, Cystatin C, GFR, and RBF but not with urinary tubular markers (NAG, NGAL, and KIM-1).

Table 2 shows the result of multivariable linear regression analysis for pro-ENK. In the final model (R^2=0.616), higher log pro-ENK levels were associated with lower GFR (standardized beta = -0.377) higher BUN, higher NT-proBNP, lower NYHA class, and lower systolic blood pressure.

Table 1. Baseline characteristics and relationship between tertiles of pro-ENK in renal mechanistic cohort

Variables	All cohort (n=95)	Terile 1 (n=32)	Tertile 2 (n=31)	Tertile 3 (n=32)	P value
pro-ENK (median, [min-max], pmol/mL)	62.2 [29.3-306.6]	45.7 [29.3-53.2]	62.2 [53.5-75.5]	102.5 [76.1-306.6]	
Age (yrs)	60±12	56±11†	61±11	63±12	0.034
Male (%)	75 (79)	27 (84)	28 (90)‡	20 (63)	0.017
Body surface area (m2)	2.0±0.2	2.1±0.2†	2.0±0.2	1.9±0.2	0.003
Systolic blood pressure (mmHg)	120±21	130 ±20†	121 ±19‡	109 ±20	<0.001
Diastolic blood pressure (mmHg)	69±12	75 ±10†	72 ±11‡	61 ±11	<0.001
Heart rate (bpm)	65±13	64 ±11	65 ±15	67 ±12	0.815
Ischemic etiology (%)	52 (55)	15 (47)	20 (65)	17 (53)	0.363
Diabetes (%)	13 (14)	6 (19)	2 (7)	5 (16)	0.338
Smoking current or Ex (%)	47 (49)	13 (43)	19 (63)	15 (52)	0.297
NYHA III or IV (%)	34 (36)	4 (13)†	11 (36)	19 (59)	<0.001
LVEF (%)	29±10	30±9†	28±10	27±10	0.441
Medication					
ACE-I (%)	78 (82)	27 (84)	27 (87)	24 (75)	0.419
ARB (%)	18 (19)	5 (16)	5 (16)	8 (25)	0.562
Beta blocker (%)	80 (84)	27 (84)	26 (84)	27 (84)	0.998
Mineralocorticoid Receptor Antagonist (%)	28 (30)	8 (25)	5 (16)‡	15 (47)	0.022
Diuretics (%)	63 (66)	18 (56)	18 (58)	27 (84)	0.029
Hemoglobin (g/dL)	14.0±1.4	14.3±1.1†	14.0±1.0‡	13.2±1.6	0.001
Hematocrit (%)	42±4	42±3†	43±3‡	39±5	0.001

NT-ProBNP (ng/L)	854.0 (287.8-1911.5)	370.4† (204.6-829.6)	431.1‡ (218.3-1210.0)	1973.0 (1186.3-3214.0)	<0.001
Renal function					
Creatinine (mg/dL)	1.2 (1.0-1.4)	1.0 (1.0-1.2)†	1.1 (1.1-1.3)‡	1.5 (1.2-1.8)	<0.001
BUN (mg/dL)	20.4 (16.7-29.1)	16.7 (14.2-19.2)*†	19.9 (18.4-22.4)‡	33.2 (24.7-41.5)	<0.001
Cystatin C (mg/L)	0.82 (0.70-1.02)	0.69 (0.49-1.78)*†	0.81 (0.59-1.12)‡	1.19 (0.64-2.09)	<0.001
GFR (mL/min/1.73m2)	72.4±27.9	92.2±23.4*†	77.2±15.2‡	47.2±22.5	<0.001
RBF (mL/min/1.73m2)	450.1±162.2	563.3±145.2*†	470.7±94.4‡	307.5±138.4	<0.001
FF (%)	28.0 (25.0-29.9)	28.4 (25.8-30.1)	28.3 (26.6-29.2)	26.5 (20.4-29.6)	0.122
Urinary KIM-1 (ng/gCr)	354.6 (218.4-604.7)	386.9 (219.7-536.7)	276.5 (207.5-630.1)	305.2 (220.3-549.2)	0.909
Urinary NAG (U/gCr)	12.9 (6.5-16.9)	13.3 (6.0-17.2)	10.3 (6.5-12.8)‡	15.0 (13.1-19.6)	0.035
Urinary NGAL (μg/gCr)	177.6 (61.1-341.8)	152.8 (52.7-314.0)	187.7 (79.9-329.4)	153.2 (57.5-366.5)	0.563
Urinary Creatinine (mmol/L)	6.2 (4.6-8.4)	7.4 (5.1-9.4)	6.3 (4.6-8.0)	6.1 (4.6-7.7)	0.391
Urinary Albumin (mg/L)	5.4 (3.3-11.8)	4.8 (2.2-8.9)†	4.8 (3.2-7.0)‡	15.0 (4.6-41.0)	0.003

† P < 0.05, Tertile 1 vs Tertile 2

‡ P < 0.05, Tertile 1 vs Tertile 3

§ P < 0.05, Tertile 2 vs Tertile 3

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BUN, blood urea nitrogen; GFR, glomerular filtration rate; KIM-1, kidney injury molecule 1; LVEF, left ventricular ejection fraction; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; pro-ENK , proenkephalin

Table 2. Multivariable linear regression for pro-ENK in renal mechanistic and acute heart failure cohort

Multivariate linear regression for Log PENK			
Variables	Standardized Beta	t	P value
Renal mechanistic cohort (Adjusted R^2=0.616)			
GFR per BSA	-0.377	-3.189	0.002
Log BUN	0.321	2.996	0.004
Log NT-proBNP	0.284	3.092	0.003
NYHA III or IV	-0.245	-2.617	0.010
Systolic blood pressure	-0.197	-2.845	0.005
Acute heart failure cohort (Adjusted R^2=0.469)			
Creatinine	0.445	14.30	<0.001
Male	-0.21	-9.647	<0.001
Age	0.163	7.583	<0.001
BNP	0.147	6.899	<0.001
BUN	0.119	3.819	<0.001
Hemoglobin	-0.113	-5.273	<0.001
BMI	-0.097	-4.507	<0.001
Glucose	-0.085	-4.167	<0.001
Potassium	0.077	3.687	0.002
Uric acid	0.056	2.442	0.015

BMI, body mass index; BNP; brain natriuretic peptide; BUN, blood urea nitrogen; GFR, glomerular filtration rate; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; pro-ENK, proenkephalin

Acute heart failure cohort

Patient characteristics

Baseline characteristics of the PROTECT AHF cohort according to pro-ENK tertiles are shown in Table 3. The mean age was 71±11 years, 1049 (66%) were male, and mean LVEF was 33±13%. The median value of pro-ENK was 104.9 (IQR: 73.7 – 146.6) pmol/L (Figure 1), and 1092 (68.7%) patients had pro-ENK levels above 99th percentile upper reference limit of pro-ENK in healthy subjects. At baseline, higher pro-ENK levels were associated with higher age, females, lower diastolic blood pressure, preserved LVEF (≥45%), history of diabetes, higher creatinine, higher BUN, and higher brain natriuretic peptide (BNP).

Table 3. Baseline characteristics according to tertiles of pro-ENK in acute heart failure (PROTECT) cohort

Variables	All cohort (n=1589)	Tertile 1 (n=530)	Tertile 2 (n=529)	Tertile 3 (n=530)	P value
pro-ENK (median, [min-max])	104.9 [6.5-511.7]	64.4 [6.5-82.9]	104.9 [83.0-131.8]	173.5 [131.9-511.7]	
Age (years)	71±11	67±11†‡	71±11§	74±10	<0.001
Male (%)	1049 (66)	379 (72)‡	343 (65)	327 (62)	0.003
Systolic blood pressure (mmHg)	125±17	126±17	123±18	125±18	0.067
Diastolic blood pressure (mmHg)	74±12	76±11†‡	73±12	72±12	<0.001
Pulse rate (bpm)	80±16	82±15†	80±15	78±16	0.001
Assigned to Rolofylline (%)	1065 (67)	352 (66)	354 (67)	359 (68)	0.899
LVEF (%)	32±13	31±13‡	31±13§	35±13	0.002
HFpEF (LVEF ≥45%) (%)*	152 (20)	35 (15)‡	49 (19)	68 (26)	0.011
Prior medication (%)					
ACE-I	993 (63)	352 (66)‡	333 (63)	308 (58)	0.019
ARB	241 (15)	60 (11)†‡	91 (17)	90 (17)	0.01
Beta blocker	1196 (75)	394 (74)	404 (77)	398 (75)	0.707
Calcium channel blocker	225 (14)	59 (11)‡	69 (13)	97 (18)	0.002
Aldosterone inhibitor	718 (45)	263 (50)‡	240 (46)	215 (41)	0.012
Digoxin	457 (29)	177 (33)‡	171 (32)§	109 (21)	<0.001
Past history (%)					
Hypertension	1272 (80)	414 (78)	419 (79)	439 (83)	0.132
Diabetes	733 (46)	234 (44)	233 (44)	266 (50)	0.072

Smoking	310 (20)	118 (22)	103 (20)	89 (17)	0.079
Heart failure hospitalization	782 (49)	234 (44)†	280 (53)	268 (51)	0.013
Atrial fibrillation	860 (54)	266 (51)	303 (57)	291 (55)	0.078
Worsening renal function (%)	371 (23)	99 (19)‡	115 (22)§	157 (30)	<0.001
Biomarkers					
WBC count (x10^9/L)	7.42 (6.04-9.22)	7.43 (6.25-9.05)	7.40 (6.13-9.15)	7.42 (5.84-9.40)	0.929
Hemoglobin (g/dL)	12.5 (11.2-13.8)	13.1 (11.9-14.4)†‡	12.7 (11.4-13.8)§	11.7 (10.6-12.9)	<0.001
Total cholesterol (mg/dL)	141 (117-173)	149 (123-178)†‡	139 (116-168)	136 (114-167)	0.001
Triglycerides (mgl/dL)	88 (65-122)	95 (72-126)†‡	82 (61-120)	84 (63-120)	<0.001
Albumin (mgl/dL)	3.8 (3.6-4.1)	3.9 (3.6-4.2)‡	3.9 (3.6-4.1)§	3.8 (3.5-4.1)	<0.001
BUN (mg/dL)	30 (22-41)	22 (18-28)†‡	29 (23-38)§	42 (32-56)	<0.001
Creatinine (mg/dL)	1.4 (1.1-1.8)	1.1 (0.9-1.3)†‡	1.4 (1.2-1.6)§	1.8 (1.5-2.3)	<0.001
NGAL (ng/mL)	82.4 (52.8-135.1)	56.6 (39.8-82.9)†‡	75.8 (53.9-112.5)§	132.8 (87.8-198.8)	<0.001
Sodium (mEq/L)	140 (137-142)	140 (137-143)‡	140 (137-142)	139 (137-142)	0.013
Potassium (mEq/L)	4.2 (3.9-4.6)	4.1 (3.8-4.5)‡	4.2 (3.8-4.6)§	4.3 (3.9-4.8)	<0.001
Glucose (mg/dL)	128 (103-164)	133 (106-175)†‡	124 (101-162)	126 (101-160)	0.008
Uric acid (mg/dL)	8.8 (7.2-10.6)	7.9 (6.6-9.5)†‡	8.9 (7.3-10.6)§	9.6 (7.9-11.6)	<0.001
BNP (pg/mL)	449.2 (255.9-801.5)	319.3†‡ (201.6-556.6)	510.9 (277.8-854.9)	542 (293.9-968.9)	<0.001
C-reactive protein (ng/mL)	13844 (7271-27939)	13683 (6978-27339)	13307 (6956-27048)	14707 (8037-29315)	0.303

* P < 0.05, Tertile 1 vs Tertile 2

† P < 0.05, Tertile 1 vs Tertile 3

‡ P < 0.05, Tertile 2 vs Tertile 3

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; HFpEF, heart failure with preserved ejection fraction; LVEF, left ventricular ejection fraction; NGAL, Neutrophil Gelatinase-Associated Lipocalin; pro-ENK , proenkephalin; WBC, white blood cell

*LVEF data was only available in 763 (48.0%) patients.

Correlation between covariates and pro-ENK

The result of univariate and multivariable linear regression analysis of pro-ENK is shown in Supplemental Table 2 and Table 2, respectively. Serum creatinine was the primary determinant of log pro-ENK among baseline variables (standardized beta = 0.422, P<0.001), and followed by females, higher age, higher BNP, and higher BUN in PROTECT acute heart failure cohort.

Association between pro-ENK and WRF

High pro-ENK values at baseline were associated with a higher incidence of worsening renal function. In univariate logistic regression, log pro-ENK was significantly associated with worsening renal function (Odds ratio: 1.47, 95% CI: 1.18-1.84, P<0.001). However, the significance was attenuated after adjustment for log creatinine (Odds ratio: 1.24, 95% CI: 0.95-1.61, P=0.119). In a sensitivity analysis, log pro-ENK was not a significant predictor of WRF with other definitions (≥25% increase or ≥25% and ≥0.3 mg/dL increase in creatinine from baseline levels) even in a univariate logistic regression analysis (data not shown).

Association of pro-ENK with prognosis

Kaplan-Meier curves of each tertile for mortality through day 180 are shown in Figure 2. Higher tertiles of pro-ENK were associated with 180 days mortality (*P*<0.001). In Cox regression models, high log pro-ENK levels were significantly associated with all of the three outcomes; death through 180 days, heart failure rehospitalization through day 60, and death or cardiovascular or renal rehospitalization through day 60 in univariable Cox regression analysis, and even after adjustment for age and gender (Model 1). Log pro-ENK was a significant predictor only for endpoint of death through day 180 even after being adjusted by age, gender, creatinine, and BUN (Model 2). However, log pro-ENK lost its significance for all of outcomes after adjustment for the PROTECT prognostic model; including age, history of heart failure hospitalization, severity of peripheral edema, systolic blood pressure, serum sodium, BUN, creatinine, and Albumin (Model 3) (Table 4). There was no significant interaction between rolofylline treatment and prognostic predictive ability of pro-ENK for any of outcomes (all P for interaction >0.3).

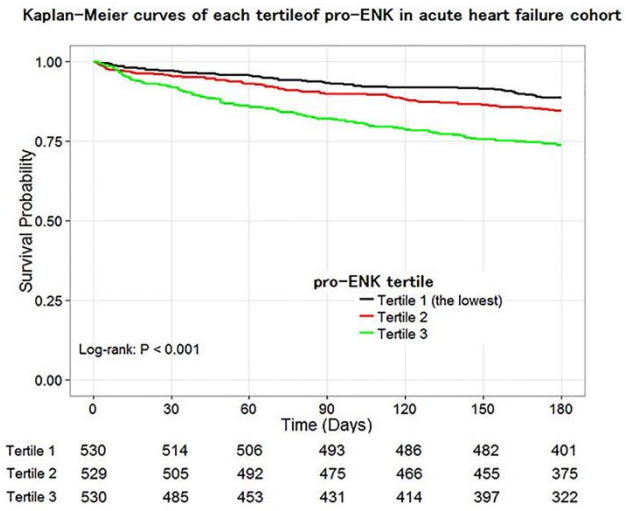

Figure 2. Kaplan-Meier curves of each tertile of pro-ENK in acute heart failure cohort
Survival curves of each pro-ENK tertile in acute heart failure cohort.

Table 4. Cox regression for outcomes in acute heart failure cohort

Outcomes	Number of Events (%)	Univariable			Model 1 (adjusted by age and gender)			Model 2 (adjusted by Model 1 + log Creatinine and log BUN)			Model 3 (adjusted by clinical model*)		
		HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
Death through Day 180	278 (17.5)	2.11	1.68-2.67	<0.001	2.02	1.59-2.57	<0.001	1.41	1.02-1.96	0.039	1.23	0.91-1.66	0.178
Heart Failure Rehospitalization through Day 60	227 (14.3)	1.43	1.12-1.84	0.005	1.53	1.18-1.97	0.001	1.01	0.72-1.38	0.933	1.01	0.74-1.36	0.977
Death or Cardiovascular or Renal Rehospitalization through Day 60	457 (28.8)	1.58	1.33-1.89	<0.001	1.63	1.36-1.96	<0.001	1.18	0.94-1.50	0.162	1.15	0.91-1.45	0.257

BUN, blood urea nitrogen

* Adjusted for age, previous heart failure hospitalization, peripheral edema, systolic blood pressure, sodium, log blood urea nitrogen, log creatinine and albumin

Serial changes in pro-ENK over time and prognosis

We compared the trajectory of pro-ENK values at day 1, day 2, and day 7 and percent change from baseline to day 2 and day 7 between patients with and without death through 180 days after excluding 29 patients who died within 7 days of admission (Figure 3). Baseline pro-ENK value was higher in patients who died compared with those who were alive. In the mixed effect model, there was no significant difference between patients who died or survived with regard to absolute or relative changes over time (*P*=0.760 and *P*=0.258, respectively). Similar results were obtained for the endpoints of heart failure rehospitalization through 60 days and death or cardiovascular or renal rehospitalization through day 60 (P>0.05 for all) (Supplemental Figure 1). We also evaluated the prognostic importance of percent change in pro-ENK from baseline (day 1) to day 2 and from baseline to day 7 as a numeric variable, and neither showed independent prognostic information in multivariate Cox regression analysis (Supplemental Table 3).

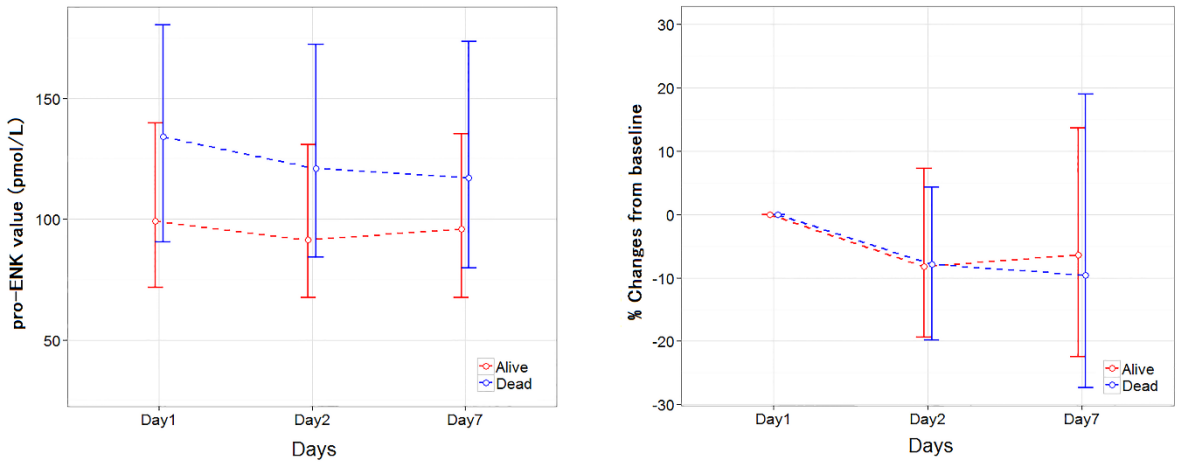

Figure 3. Changes in pro-ENK in patients with and without death through 180 days
Median value is expressed as open circle and interquartile range is expressed as error bars.

Discussion

In acute and chronic heart failure, pro-ENK levels were higher in acute HF compared with chronic HF. Pro-ENK was clearly associated with renal blood flow and glomerular filtration rate but not with tubular function. In acute heart failure patients, pro-ENK was associated with clinical outcome, but after adjustments for established prognostic predictors including preexisting renal markers, this association was lost. Therefore, pro-ENK seems to be a renal marker, but does not seem to have additive value on top of the established prognostic markers.

Pro-ENK as a renal biomarker in heart failure patients

The endogenous opioid system is one of the most studied innate pain-relieving systems. In addition, the endogenous opioid system has also been suggested to have a negative effect on the cardiovascular system. Two observational studies suggested that activity of the endogenous opioid system was activated in heart failure

patients compared with healthy subjects[16, 17]. Additionally, in an experimental dog model of congestive heart failure, delta-opioid receptor (OPR) was specified as a more relevant receptor subtype among several OPRs in terms of hemodynamic regulation[18]. In this study, a selective antagonist for delta-OPR increased aortic pressure, cardiac output, and blood flow to the myocardium and kidney. These results suggested that delta-OPR plays a main role in the opioid system as a cardiovascular modulator, and measuring activity of enkephalin - a specific peptide to delta-OPR - might be useful to evaluate the effect of the opioid system in heart failure patients. Recently, pro-ENK was suggested as a stable and reliable surrogate marker of enkephalin and it became possible to evaluate enkephalin activity in vivo[5].

In the present study, we showed that levels of pro-ENK were relatively high in both acute and chronic heart failure patients when pro-ENK value derived from normal subject was used as reference. Furthermore, both in the chronic and acute heart failure cohorts we found a consistent association between pro-ENK and several renal markers. Moreover, precise evaluation of renal function in the chronic heart failure cohort showed that pro-ENK levels were strongly associated with renal blood flow and glomerular filtration rate. These results are in agreement with the finding that delta-OPR was highly expressed in the kidney and inhibition of delta-OPR increased kidney blood flow in an experimental heart failure model[4, 18]. Moreover, pro-ENK was positively correlated with albuminuria in the chronic heart failure cohort. These findings show that pro-ENK is a novel renal marker. The pathophysiologic mechanism or rather determinants of pro-ENK including renal clearance has to be evaluated in future studies.

We evaluated the association between pro-ENK and worsening renal function in acute heart failure, and found that pro-ENK was not a predictor of worsening renal function in heart failure patients independent from serum creatinine. This is in line with a previous study that evaluated the association between pro-ENK values before surgery and acute kidney injury in patients undergoing cardiac surgery. In this study, baseline pro-ENK values were strongly associated with baseline creatinine. Pro-ENK levels were also associated with acute kidney injury after surgery, but did not outweigh creatinine[6]. These and our results showed that the association between pro-ENK and worsening renal function can be attributed to the significant association with creatinine, and pro-ENK by itself provides limited additive information to creatinine in terms of changes in renal function.

Prognostic information of pro-ENK in heart failure

In our present analysis, pro-ENK was not an independent predictor of prognosis in acute heart failure cohort in spite of its association with renal function and severity of heart failure. This result suggests that pro-ENK provides limited additional prognostic information to preexisting prognostic markers of heart failure patients including renal biomarkers.

Our findings are inconsistent with previous two studies which investigated prognostic role of pro-ENK in patients with myocardial infarction and non-symptomatic heart failure patients, where higher pro-ENK levels were an independent predictor of a combined endpoint of death and adverse events even after adjustment for other prognostic factors[7, 19]. This discordance might be due to a difference in study population. Another possible explanation is an association between pro-ENK and BUN. In the aforementioned study of myocardial infarction patients, pro-ENK was an independent predictor of mortality after being adjustment for the Global Registry of Acute Coronary Events (GRACE) model[20], and pro-ENK showed incremental prognostic information. However, the

GRACE model does not include information about BUN, and as a consequence, it is unclear whether pro-ENK would have been a significant predictor of events if the model would have been adjusted for BUN. Recent studies showed that BUN was an independent predictor of mortality also in acute myocardial infarction patients even after being adjusted by eGFR[21, 22] and indeed pro-ENK was significantly and strongly correlated with BUN in our cohort. Recently, Arbit et al. investigated the role of pro-ENK in patients referred to echocardiography and categorized into stage A or B HF (symptomatic HF patients were excluded). Pro-ENK correlated with serum creatinine and eGFR, and was an independent predictor of worse prognosis after adjustment for some prognostic factors. However, in contrast to the present study, these patients were asymptomatic and were not adjusted for BUN, which was a strong confounder in our study[19]. The relationship between pro-ENK and BUN might be an explanation why pro-ENK was an independent prognostic predictor in these previous studies but not in our cohort.

Limitations

This study has important limitations due to its retrospective character. In the chronic HF cohort, number of patients were limited so that prognostic predictability of pro-ENK in a chronic heart failure population remains to be elucidated. In the acute heart failure cohort, only heart failure patients with mild renal impairment were included by study design. Echocardiographic measurements were obtained in only less than half of all patients. Moreover, pro-ENK levels were not available in some patient of both cohorts due to availability of plasma, which could have influenced the results despite the fact that there was no significant difference in event rate for any endpoints between patients with available samples and those without (all P value >0.5).

Conclusion

Pro-ENK levels were higher in acute heart failure when it compared with chronic heart failure. Pro-ENK levels were strongly associated with glomerular function and renal blood flow, but not with tubular damage. Pro-ENK has limited additive prognostic predictive information on top of existing renal markers in this cohort of acute heart failure.

Funding: The PROTECT trial was supported by NovaCardia, a subsidiary of Merck. Alere, Singulex, and Sphingotec kindly provided assays and performed biomarker measurements.

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Supplemental materials

Supplemental Table 1. Univariate linear regression for pro-ENK in renal mechanistic cohort

Variables	Standardized Beta	t	P value
Age	0.29	2.922	0.004
Male	-0.257	-2.566	0.012
BSA	-0.362	-3.727	<0.001
Systolic blood pressure	-0.375	-3.897	<0.001
Diastolic blood pressure	-0.475	-5.201	<0.001
Heart rate	0.008	0.081	0.936
Ischemic etiology	0.012	0.115	0.909
Diabetes	-0.029	-0.279	0.781
Smoking current or Ex	0.048	0.449	0.655
NYHA III or IV	0.417	4.421	<0.001
LVEF	-0.134	-1.301	0.197
Medication			
ACE-I	-0.119	-1.158	0.25
ARB	0.109	1.062	0.291
Beta blocker	-0.025	-0.246	0.806
Aldosterone antagonist	0.326	3.321	0.001
Diuretics	0.27	2.701	0.008
Hemoglobin	-0.34	-3.452	<0.001
Hematocrit	-0.326	-3.213	0.002
Log NT-ProBNP	0.588	7.006	<0.001
Renal function			
Creatinine	0.606	7.349	<0.001
Log BUN	0.714	9.794	<0.001
Cystatin C	0.706	8.628	<0.001
GFR	-0.707	-9.590	<0.001
RBF	-0.66	-7.300	<0.001
FF	-0.328	-3.353	0.002
Urinary KIM-1	-0.061	-0.500	0.619
Urinary NAG	0.17	1.421	0.16
Urinary NGAL	0.197	1.654	0.103
Urinary Albumin	0.309	3.063	0.003

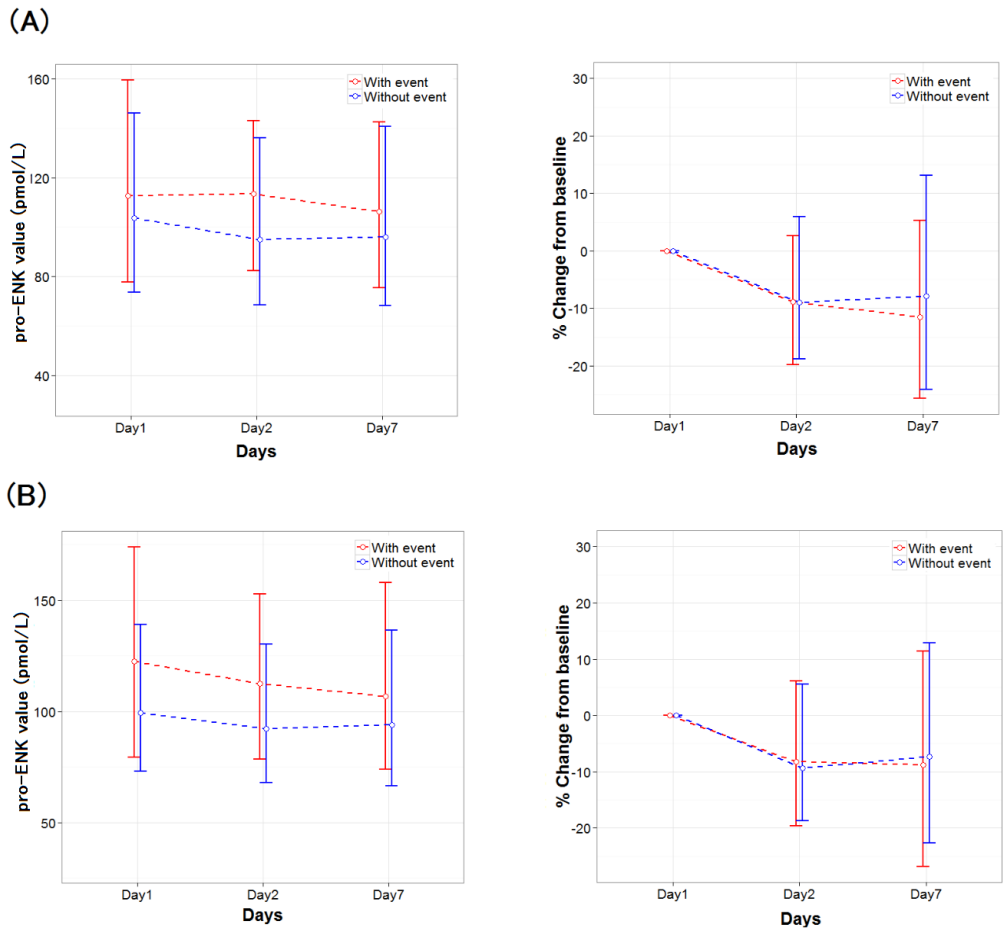
Supplemental Table 2. Univariate linear regression for pro-ENK in acute heart failure cohort

Variables	Standardized Beta	t	P value
Age	0.248	10.19	<0.001
Male gender	-0.095	-3.814	<0.001
Systolic blood pressure	-0.016	-0.633	0.527
Diastolic blood pressure	-0.091	-3.629	<0.001
Pulse rate (bpm)	-0.065	-2.609	0.009
LVEF	0.109	3.021	0.003
Prior medication			
ACE-I	-0.063	-2.533	0.011
ARB	0.039	1.558	0.119
Beta blocker	-0.001	-0.039	0.969
Calcium channel blocker	0.097	3.898	<0.001
Aldosterone inhibitor	-0.063	-2.53	0.012
Digoxin	-0.088	-3.542	<0.001
Past history			
Hypertension	0.051	2.035	0.042
Diabetes	0.056	2.244	0.025
Smoking	-0.075	-2.812	0.005
Heart failure hospitalization	0.064	2.553	0.011
Atrial fibrillation	0.049	1.942	0.052
Biomarkers at baseline			
WBC	0.007	0.275	0.783
Hemoglobin	-0.269	-10.48	<0.001
T-Cholesterol	-0.001	-2.528	0.012
Triglycerides	-0.041	-1.598	0.11
Albumin	-0.156	-4.819	<0.001
BUN	0.517	23.87	<0.001
Creatinine	0.552	26.09	<0.001
Plasma NGAL	0.299	12.41	<0.001
Sodium	-0.06	-2.374	0.018
Potassium	0.163	6.358	<0.001
Glucose	-0.075	-2.924	0.004
Uric acid	0.232	9.228	<0.001
BNP	0.245	10.05	<0.001
C-reactive protein	0.04	1.589	0.112

Supplemental Table 3. Univariable and multivariable Cox regression of percent change in pro-ENK value from baseline to day2 and day7 for outcomes (per 10% increase)

Outcomes	Percent relative changes from Day 1 to Day 2						Percent relative changes from Day 1 to Day 7					
	Univariable Cox			Multivariate Cox			Univariable Cox			Multivariate Cox		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
Death through Day 180	1.01	0.99-1.02	0.353				1.00	0.99-1.01	0.638			
HF Rehospitalization through Day 60	1.01	1.00-1.02	0.030	1.01	1.00-1.02	0.157	1.01	1.00-1.02	0.044	1.01	0.99-1.02	0.279
Death or Cardiovascular or Renal hospitalization through Day 60	1.09	0.99-1.01	0.395				1.01	0.99-1.01	0.107			

Supplemental Figure 1. Changes in pro-ENK in patients with and without (A) heart failure rehospitalizaiton through day 60 and (B) death or cardiovascular or renal rehospitalization through day 60



Chapter 3

Blood urea nitrogen to creatinine ratio in the general population and in patients with acute heart failure

Yuya Matsue
Peter van der Meer
Kevin Damman
Marco Metra
Christopher M. O'Connor
Piotr Ponikowski
John R. Teerlink
Gad Cotter
Beth Davison
John G. Cleland
Michael M. Givertz
Daniel M. Bloomfield
Howard C. Dittrich
Ron T. Gansevoort
Stephan J.L. Bakker
Pim van der Harst
Hans L. Hillege
Dirk J. van Veldhuisen
Adriaan A. Voors

Heart 2017;103:407-413

Abstract

Objective

The blood urea nitrogen (BUN)/Creatinine ratio has been proposed as a useful parameter in acute heart failure (AHF), but data on the normal range and the added value of the ratio compared to its separate components in patients with AHF are lacking. The aim of this study is to define the normal range of BUN/Creatinine ratio and to investigate its clinical significance in patients with AHF.

Methods

In 4484 subjects from the general population without cardiovascular comorbidities, we calculated age and sex specific normal values of the BUN/Creatinine ratio, deriving a higher and lower than normal range of BUN/Creatinine ratio (exceeding the 95% prediction intervals). Association of abnormal range to prognosis was tested in 2033 AHF patients for the outcome of all-cause death through 180 days, death or cardiovascular or renal rehospitalization through 60 days, and heart failure rehospitalization within 60 days.

Results

In a cohort of AHF patients, 482 (24.6%) and 28 (1.4%) HF patients were classified into higher and lower than normal range groups, respectively. In Cox regression analysis, higher than normal range of BUN/Creatinine ratio group was an independent predictor for all-cause death (HR: 1.86, 95% CI: 1.29-2.66) and death or cardiovascular or renal rehospitalization (HR: 1.37, 95% CI: 1.03-1.82), but not for heart failure rehospitalizaiton (HR: 1.23, 95% CI: 0.81-1.86) after adjustment for other prognostic factors including both creatinine and BUN.

Conclusions

In AHF patients, BUN/Creatinine higher than age and sex specific normal range is associated with worse prognosis independently from both creatinine and BUN.

Keywords: Heart Failure; Blood urea nitrogen; Kidney; Prognosis

Introduction

Renal dysfunction is one of the most common comorbidities in acute heart failure (HF) and it is related to poor prognosis[1]. Creatinine and blood urea nitrogen (BUN) are nitrogenous end products of protein metabolism, and freely filtered at the glomerulus because both are relatively small molecules. Therefore, both serum creatinine and BUN are well recognized as renal markers and have been shown to be associated with outcome in these patients[1, 2]. However, there is a difference in tubular handling between these two renal markers: while creatinine is but not reabsorbed, approximately 40 to 50% of BUN is reabsorbed in the tubules. As this reabsorption process is directly or indirectly regulated by neurohormonal activity[3], the BUN to Creatinine (BUN/Creatinine) ratio has been proposed as a metric of neurohormonal activity which may have prognostic value in HF[4-10]. However, normal values of BUN/Creatinine ratio are unknown and therefore qualitative evaluation and use of BUN/Creatinine has been limited by a lack of reference values. To better understand the distribution, etiology, and prognostic implication of BUN/Creatinine ratio, we set out to establish normal values of BUN/Creatinine ratio in the general population. Subsequently, we applied these values to a cohort of acute HF patients.

Methods

The Prevention of Renal and Vascular End-stage Disease (PREVEND) study cohort was used to investigate BUN/Creatinine ratio in general population. From these normal values, we derived a normal range, which was applied on a cohort of 2033 patients from the Placebo-controlled Randomized study of the selective A1 adenosine receptor antagonist rolofylline for the patients hospitalized with AHF and volume Overload to assess Treatment Effect on Congestion and renal functTion (PROTECT) study cohort.

PREVEND cohort (general population)

The PREVEND study was designed to prospectively investigate the natural course of increased levels of urinary albumin excretion and its relation to renal and CV disease in a large cohort drawn from the general population. Details of this protocol and results have been described elsewhere[11, 12]. In brief, all inhabitants of the city of Groningen, The Netherlands, aged 28 to 75 years (N=85,421) were asked to send in a first morning urine sample and complete a short questionnaire on demographics and cardiovascular disease history. Of these subjects, 40,856 responded (47.8%). After exclusion with insulin dependent diabetes mellitus and pregnant women, 6,000 subjects with urinary albumin excretion ≥10mg/L in their morning urine and randomly selected 2,592 subjects with urinary albumin excretion <10mg/L were further investigated in an outpatient clinic. These 8,592 subjects constitute the PREVEND cohort. BUN/Creatinine ratio was obtained in 7976 (92.8%) patients. In order to investigate BUN/Creatinine ratio in a cohort from the general population without cardiovascular comorbidities, we excluded 3492 subjects from this cohort with a history of hypertension, hypercholesterolemia, diabetes or myocardial infarction.

Excluded subjects were older, often male, and more often had a history of smoking compared to included subjects. All of the biomarkers, including creatinine and BUN, were higher in excluded subjects, and there was a small but

significant difference in BUN/Creatinine ratio between the included and excluded subjects (Supplemental Table 1). The remaining 4484 subjects were used for the present analysis.

Creatinine was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY), an automated enzymatic method. BUN was measured from samples that were kept stored frozen at -80 degrees Centigrade from +/- 1997 until 2012. The BUN measurements were performed on a Roche Modular with UV kinetic assay, which is based on Talke and Schubert's method and has been optimized for analyzers that permit kinetic measurements.

All subjects gave written informed consent. The PREVEND study was approved by the local medical Ethical Committee and conducted in accordance with the Declaration of Helsinki.

PROTECT cohort (acute heart failure patients)

We evaluated the prognostic significance of BUN/Creatinine ratio in the PROTECT study cohort. Study design, primary results, and conclusions have been already published[13-15]. In brief, 2,033 patients with acute HF and renal function impairment (estimated creatinine clearance between 20 to 80 mL/min) were included and randomized to rolofylline or placebo. The protocol of the PROTECT study was approved by the ethics committee at each participating center, and written informed consent was obtained from all participants. BUN/Creatinine ratio was obtained in 1956 (96.2%) patients at baseline, and we divided the cohort into three groups according to upper/lower limits of 95% prediction intervals of BUN/Creatinine ratio calculated from the equation derived from the PREVEND cohort. An estimate of the glomerular filtration rate was calculated using the simplified Modification of Diet in Renal Disease (sMDRD) formula. Both serum BUN and creatinine were measured in a central laboratory (ICON Laboratories, NY). Creatinine was measured using substrate-triggered rate-blanked method.

Worsening renal function (WRF) was defined as a creatinine increase of ≥0.3 mg/dL from baseline (day 1) value or initiation of hemofiltration or dialysis at any time between day 1 to day 4. The prognostic implication of lower and higher than normal range of BUN/Creatinine ratio was evaluated using three endpoints: all-cause mortality within 180 days, death or cardiovascular or renal rehospitalization through day 60 days, and HF rehospitalization through 60 days[16].

Statistical analysis

Data are expressed as mean and standard deviation for normally distributed variables, and as median with interquartile range for non-normally distributed data. Categorical data are expressed as numbers and percentages. In the PREVEND study, patients with elevated urinary albumin excretion were overselected compared to those without. To overcome this limitation, a design-based analysis (statistical weighting method) was performed so that we can generalize our results to general population[17].

The relationship between baseline characteristics and each BUN/Creatinine group was compared by using one-way analysis of variance test, Kruskal-Wallis test, or chi-squared tests where appropriate. When necessary, variables were transformed for further analyses. Logistic regression was performed to evaluate the predictability of higher/lower than normal range of BUN/Creatinine ratio for WRF. For prognostic analysis, the hazard ratio of being higher/lower than normal range of BUN/Creatinine ratio group was adjusted by the clinical model previously

defined for this cohort in Cox regression model[18]. The prognostic predictability of this clinical model was confirmed to be similar to more complex models for the outcome of all-cause mortality within 180 days, death or rehospitalization for any reason within 30 days, and death or cardiovascular or renal rehospitalization within 30 days in PROTECT cohort[18]. The proportional hazards assumption of Cox regression was tested by analysis of the scaled Schoenfeld residuals. For the variables did not meet this assumption, stratification was performed. For the outcome of heart failure rehospitalization through day 60, Fine and Gray competing risk proportional hazard regression model was used[19]. We also calculated continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) with corresponding 95% confidence interval for combined logistic model of aforementioned clinical model and BUN/Creatinine ratio in relation to normal range (higher, within, and lower than normal range)[20]. A two-tailed P value < 0.05 was considered statistically significant. Statistical analysis were performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). ISBN 3-900051-07-0, URL http://www.R-project.org.

Results

BUN/Creatinine ratio in general population

The median BUN/Creatinine ratio in the 4484 subjects without cardiovascular comorbidities from the general population without cardiovascular risk factor was 15.0 (IQR: 12.9-17.6) (Figure 1). After evaluating linearity, we constructed a linear regression model for the association between age and log BUN/Creatinine ratio for males and females separately, because there was a significant interaction between sex and age on log BUN/Creatinine ratio (P for interaction <0.001).

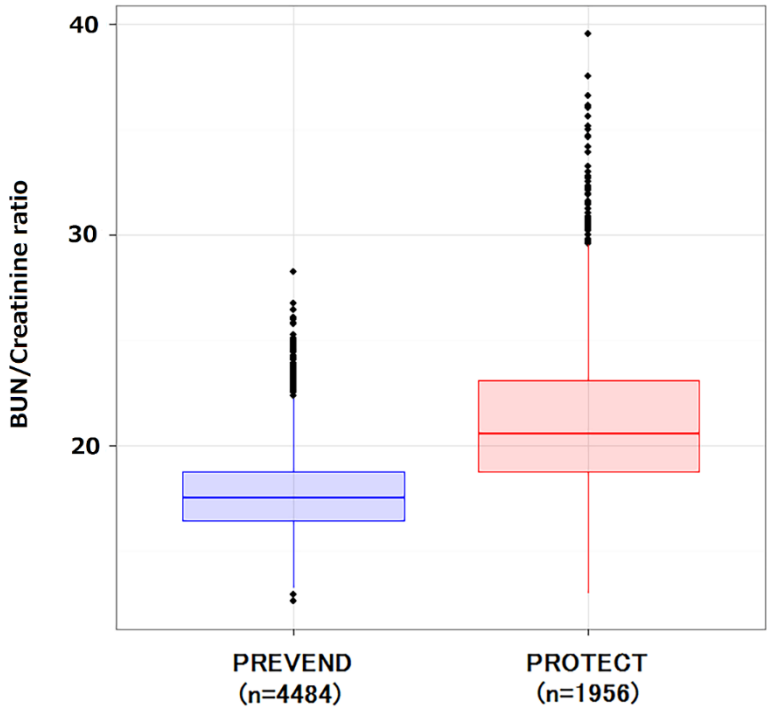


Figure 1. Baseline BUN/Creatinine ratio in general population (PREVEND) and acute heart failure patients (PROTECT)

Figure 2 shows the distribution of log BUN/Creatinine ratio in 4484 PREVEND subjects, and the regression line with 95% prediction intervals by age for each sex. Log BUN/Creatinine varied widely and increased with age in both sexes. Log BUN/Creatinine ratio increased more with age in females compared to males.

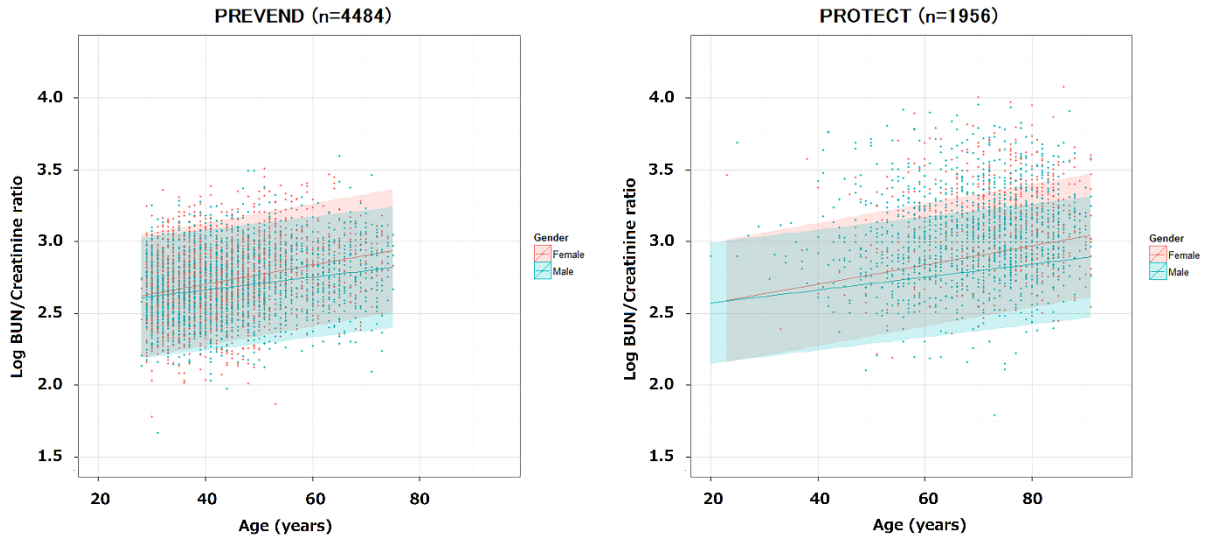


Figure 2. Scatter plot of association between age versus log BUN/Creatinine ratio by sex in general population (PREVEND) and acute heart failure patients (PROTECT)
Solid lines express predicted log BUN/Creatinine ratio by age and sex with 95% prediction intervals (shaded area) for each sex.

We additionally checked the association between age and creatinine and between age and BUN. Both creatinine and BUN increased with age both in males and females however, a significant interaction between age and sex was observed for BUN only (P for interaction=0.017) and not for creatinine (P for interaction=0.350) (Supplemental Figure 1).

BUN/Creatinine ratio in acute heart failure cohort

In the PROTECT study cohort, median BUN/Creatinine ratio was 21.1 (IQR: 17.5-26.2) which was significantly higher than in the control cohort without cardiovascular comorbidities (P<0.001) (Figure 1). The upper and lower 95% prediction limits were calculated from age and sex for each patient, and all patients were divided into three groups: higher than normal range of BUN/Creatinine ratio group (n=482; 24.7%), lower than normal range of BUN/Creatinine ratio group (n=28; 1.4%), and BUN/Creatinine ratio within normal range (n=1446; 73.9%). The baseline characteristics of each group are described in Table 1. At baseline, higher than normal range of BUN/Creatinine ratio was associated with lower age, higher BUN, higher NGAL, lower blood pressure, lower left ventricular ejection fraction, a HF hospitalization in the previous year, and lower plasma sodium level. There was no significant difference in either creatinine or estimated glomerular filtration rate between the groups.

Table 1. Baseline characteristics of each BUN/Creatinine ratio group in acute heart failure patients (PROTECT)

Variables	BUN/Creatinine ratio higher than normal range (n=482)	BUN/Creatinine ratio within normal range (n=1446)	BUN/Creatinine ratio lower than normal range (n=28)	P value
Age (years)	69±12	71±11	70±12	0.009
Male gender (%)	342 (71)	948 (66)	17 (61)	0.073
Systolic blood pressure (mmHg)	118±17	126±17	134±19	<0.001
Diastolic blood pressure (mmHg)	71±12	75±12	79±10	<0.001
Pulse rate (bpm)	79±15	81±16	81±14	0.046
Assign to Rolofylline (%)	326 (68)	962 (67)	19 (68)	0.899
LVEF* (%)	31±13	33±13	36±11	0.067
HFpEF (LVEF ≥45%) (%)	41 (17)	134 (20)	5 (33)	0.282
Prior medication (%)				
ACE-I	284 (59)	915 (63)	16 (57)	0.194
ARB	96 (20)	205 (14)	3 (11)	0.008
Beta blocker	380 (79)	1095 (76)	22 (79)	0.377
Calcium channel blocker	45 (9)	214 (15)	4 (14)	0.009
Aldosterone antagonist	256 (53)	602 (42)	7 (25)	<0.001
Digoxin	152 (32)	402 (28)	4 (14)	0.071
Past history (%)				
Hypertension	348 (72)	1180 (82)	25 (89)	<0.001
Diabetes	236 (49)	644 (45)	10 (36)	0.141
Smoking	105 (22)	289 (20)	7 (25)	0.588
Heart failure hospitalization	276 (57)	681 (47)	13 (46)	0.001
Atrial fibrillation	274 (57)	772 (54)	12 (43)	0.196
Worsening renal function (%) (≥0.3mg/dL from baseline)	111 (23)	342 (24)	9 (32)	0.543
BUN/Creatinine ratio	30.9 (27.8-35.6)	19.4 (16.7-22.5)	10 (9.9-11.2)	<0.001

eGFR (mL/min/1.73m2)	46 (33-60)	46 (35-60)	48 (38-59)	0.898
Biomarkers				
WBC count (x10^9/L)	7.26 (5.89-8.90)	7.50 (6.15-9.33)	7.56 (5.99-9.25)	0.167
Hemoglobin (g/dL)	12.4±2.0	12.8±2.0	12.0±2.1	<0.001
Total cholesterol (mg/dL)	26 (105-156)	147 (120-177)	159 (139-190)	<0.001
Triglycerides (mgl/dL)	82 (62-113)	90 (65-126)	106 (83-132)	0.003
Albumin (mgl/dL)	3.8 (3.5-4.1)	3.9 (3.6-4.1)	3.8 (3.3-4.3)	<0.001
Blood urea nitrogen (mg/dL)	45 (34-63)	27 (21, 35)	13 (11-15)	<0.001
Creatinine (mg/dL)	1.4 (1.1-1.9)	1.4 (1.1-1.7)	1.3 (1.1-1.6)	0.358
Sodium (mEq/L)	139 (136-141)	140 (137-142)	141 (139-142)	<0.001
Potassium (mEq/L)	4.4±0.6	4.3±0.6	4.2±0.5	0.027
Glucose (mg/dL)	128 (103-162)	126 (103-164)	113 (103-140)	0.534
Uric acid (mg/dL)	9.9± 2.9	8.7±2.4	7.4±1.9	<0.001
BNP (pg/mL)	529.4 (279.1-946.0)	432.9 (248.1-766.7)	303.1 (225.4-558.4)	<0.001
C-reactive protein (ng/mL)	14226 (7911-27407)	13922 (7136-27946)	16095 (9261-23582)	0.595
Plasma NGAL (ng/mL)	93.8 (57.2-151.1)	78.8 (52.2-128.4)	77.6 (55.7-124.2)	0.001

We observed 462 cases of WRF, and there was no significant difference in the incidence of WRF between groups. Neither higher (odds ratio: 0.97, 95% CI: 0.76-1.23, P=0.780) nor lower (odds ratio: 1.53, 95% CI: 0.69-3.41, P=0.300) than normal range of BUN/Creatinine ratio were significantly associated with WRF in univariate logistic regression analysis.

Kaplan-Meier curves of each group for mortality through day 180 are shown in Figure 3. Higher and lower than normal range of BUN/Creatinine ratio groups were associated with worse and better outcome compared to as predicted group, respectively (Log-rank: P<0.001). In univariate Cox regression models, higher than normal range of BUN/Creatinine ratio group was associated with all of three outcomes compared to within normal range group; death through day 180, HF rehospitalization through day 60, and death or cardiovascular or renal rehospitalization through day 60. In multivariable Cox regression models, serum albumin (for the outcome of death through day 180 and death or cardiovascular or renal rehospitalization through day 60) and age (for the outcome of death or cardiovascular or renal rehospitalization through day 60) were stratified due to violation of proportional hazard assumption. Proportional hazard assumptions were not violated in the stratified model. Higher than normal range of BUN/Creatinine ratio was associated with significantly higher risks for death through day 180 and death or cardiovascular or renal rehospitalization through day 60 in multivariable analyses, even after adjustment for the clinical model including both BUN and creatinine (Table 2). There was no significant interaction between higher than normal range of BUN/Creatinine ratio and age, gender, and eGFR for any of outcomes (all P value >0.10).

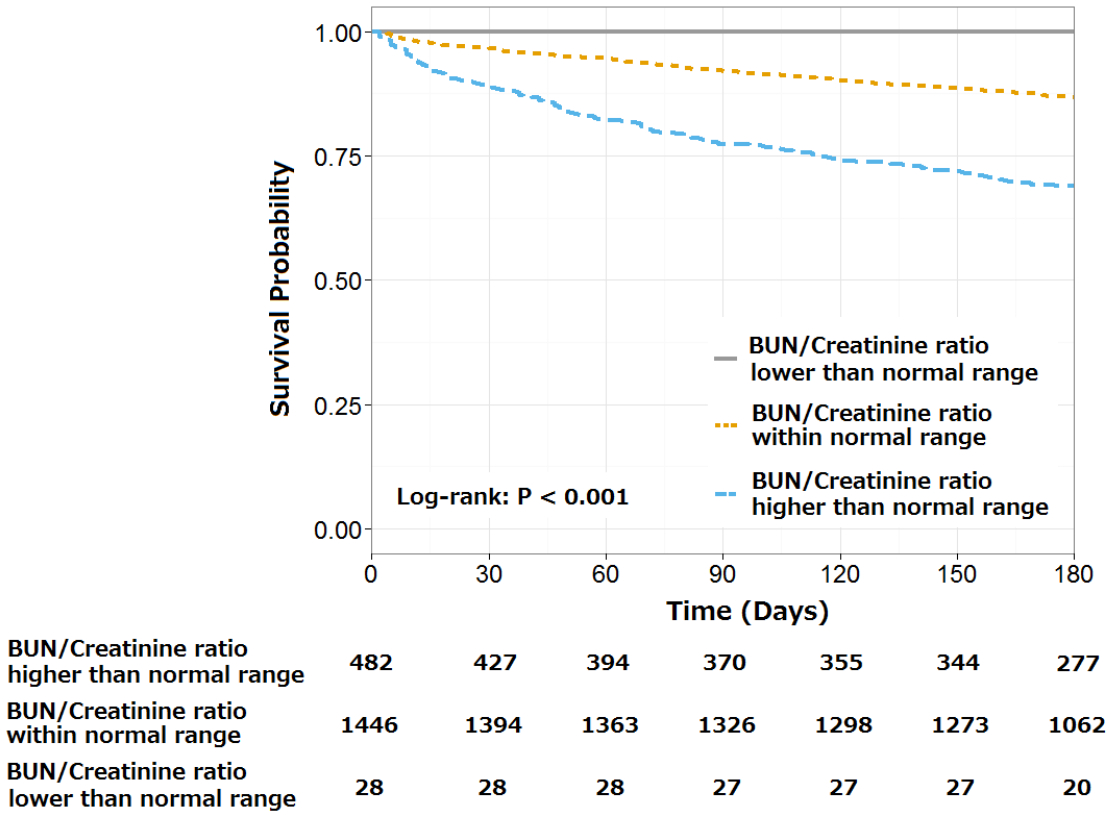


Figure 3. Kaplan-Meier survival curves for all-cause mortality within 180 days for the three different groups in acute heart failure patients (PROTECT)

Table 2. Cox regression analysis for outcomes in acute heart failure patients (PROTECT)

Outcomes	Number of Event (%)	Univariate Cox			Adjusted model*		
		HR	95%CI	P value	HR	95%CI	P value
Death through Day 180	**340 (17.4)**						
BUN/Creatinine ratio within normal range	191 (13.2)	1 (Reference)			1 (Reference)		
BUN/Creatinine ratio lower than normal range	0	-	-	-	-	-	-
BUN/Creatinine ratio higher than normal range	149 (30.9)	2.66	2.14-3.29	<0.001	1.86	1.29-2.66	<0.001
Death or Cardiovascular or Renal Rehospitalization through Day 60	**558 (28.5)**						
BUN/Creatinine ratio within normal range	360 (24.9)	1 (Reference)			1 (Reference)		

BUN/Creatinine ratio lower than normal range	5 (17.9)	0.69	0.28-1.66	0.405	0.99	0.39-2.53	0.997
BUN/Creatinine ratio higher than normal range	193 (40.0)	1.82	1.53-2.16	<0.001	1.37	1.03-1.82	0.03
HF Rehospitalization through Day 60	**285 (14.6)**						
BUN/Creatinine ratio within normal range	190 (13.1)	1 (Reference)			1 (Reference)		
BUN/Creatinine lower than normal range	2 (7.1)	0.52	0.13-2.05	0.35	0.74	0.18-3.14	0.689
BUN/Creatinine higher than normal range	93 (19.3)	1.53	1.20-1.96	<0.001	1.23	0.81-1.86	0.337

* Adjusted for age, previous heart failure hospitalization, peripheral edema, systolic blood pressure, sodium, log blood urea nitrogen, log creatinine and albumin

When BUN/Creatinine ratio group was added to clinical model, significant NRI and IDI were observed for outcome of death through 180 days (NRI: 0.27, P<0.001, and IDI: 0.01, P<0.001), death or cardiovascular or renal rehospitalization through day 60 (NRI: 0.15, P=0.003, and IDI: 0.003, P=0.016), but not for heart failure rehospitalization (NRI: 0.08, P=0.228, and IDI: 0.00, P=0.865).

The PREVEND cohort did not include subjects older than 75 years but 37.6% of the PROTECT cohort in our study were over 75 years old. We therefore performed a sensitivity analysis only for the PROTECT study cohorts aged 75 years or younger. In Cox regression analysis, higher than normal range of BUN/Creatinine ratio group was consistently associated with a higher risk for both outcomes of death through 180 days and death or cardiovascular or renal rehospitalization through day 60 in both univariate and multivariable analysis (Supplemental Table 2). P value for interaction between age above/below 75 years and BUN/Creatinine ratio group was not significant for any of outcomes (all P value > 0.50).

Discussion

In the present study, we showed several novel findings regarding BUN/Creatinine ratio both in the general population and in patients who were hospitalized for acute HF. In the general population, there was a wide variation in BUN/Creatinine ratio. BUN/Creatinine ratio increased with age and more in females compared with males. BUN/Creatinine ratio was higher in acute HF patients compared with the general population, and a quarter of them showed higher than normal range of BUN/Creatinine ratio. Higher than normal range of BUN/Creatinine ratio was associated with more severe HF symptoms, and also associated with higher mortality even after adjustment for other prognostic factors including creatinine and BUN.

BUN to creatinine ratio in normal subjects

We evaluated subjects without cardiovascular comorbidities in PREVEND and found that age and sex were significant determinants of BUN/Creatinine ratio. The age-related increase in BUN/Creatinine ratio can be partially

explained by difference in age-related change of these two biomarkers. Even if age-associated glomerular function impairment affects both creatinine and BUN equally, the magnitude of increase in creatinine, but not in BUN, might be attenuated by a decrease in muscle mass with increasing age [21]. This hypothesis is supported by the recent study which investigated the difference in age-related change in handling between creatinine and BUN[22]. In this study, only serum BUN but not creatinine increased with age, and they also showed this difference was derived primarily from smaller age-related reduction in production of BUN compared to creatinine. In other words, age-related decrease in clearance exceed age-related reduction in production only in BUN, but not creatinine, and this difference is a main driver of age-related increase in BUN/Creatinine ratio. Another study also showed the increase in BUN/Creatinine ratio with age in normal subjects[23].

We also found that there was a significant interaction between age and sex on BUN/Creatinine ratio. With increasing age, BUN/Creatinine ratio was increased more in females than males, and this sex difference in age-related increase in BUN/Creatinine ratio is derived primarily from the difference in the age-related increase in BUN levels rather than creatinine. This finding can potentially be partially explained by a sex difference in change in protein turnover with age. Two recent studied focusing on elderly females showed higher protein synthesis rate compared to males, despite the females having less muscle mass than males[24, 25]. Interestingly, this difference in protein turnover was not shown between middle-aged males and females[26]. These findings imply that sex differences in protein turnover do not occur until later in life. Indeed, high BUN/Creatinine ratio was shown in subjects who are over the age of 65 and without previously detected medical disorders[27], but not in children (mean age 12.4 years old)[28]. These findings support our hypothesis regarding sex difference in age-related change in BUN/Creatinine ratio. Although further investigations are needed to clarify the pathophysiological background of BUN/Creatinine ratio in normal subjects, our results clearly showed the importance of taking age and sex into account when we evaluate BUN/Creatinine ratio.

BUN to creatinine ratio in acute heart failure

The reabsorption process of BUN in the tubules is directly and indirectly potentiated by activation of renin-angiotensin-aldosterone activity, sympathetic nervous activity, and arginine-vasopressin activity[3]. Therefore, BUN is suggested as an integrated marker of renal function and neurohormonal activation. In fact, BUN outweighed creatinine (or estimated glomerular filtration rate) in prognostic ability and appeared to be one of the most powerful predictors of prognosis in HF patients[18, 29]. Moreover, this physiological background forms the basis of BUN/Creatinine ratio as a maker of renal neurohormonal activity and many studies have reported an association between BUN/Creatinine ratio and hemodynamics and/or prognosis independently from creatinine or estimated glomerular function in HF[4, 5] Howevere these association were not found independent of an association with BUN, suggesting that BUN may be the real driving force of the association.

In line with this pathophysiological background, the group with higher than normal range of BUN/Creatinine ratio showed associations with some indices of neurohormonal activity. In 427 patients enrolled in the DOSE and CARRESS-HF trials, low blood pressure, low ejection fraction, low sodium and high BUN levels are associated with

the above-median levels of plasma renin or plasma aldosterone of renin-angiotensin-aldosterone activity[30]. This finding supports our speculation that higher than normal range of BUN/Creatinine ratio represents highly activated neurohormonal status in AHF patients.

Higher than normal range of BUN/Creatinine ratio at baseline was not associated with WRF in our cohort. This is in contrast to the current concept of linking between RAAS activation and WRF in AHF patients. However, limited data have directly shown this association in AHF and some studies showed disassociation. Takaya et al. showed baseline BUN/Creatinine ratio in AHF was not associated with acute kidney injury within 48 hours defined by serum creatinine increase ≥0.3 mg/dL or 50% from baseline[9]. In another AHF study, the incidence of WRF was similar in AHF patients with high/low plasma renin and aldosterone activity at baseline[30]. These findings imply a complex and multifactorial pathophysiological background of WRF in AHF patients.

We found that higher than normal range of BUN/Creatinine ratio was associated with high mortality independently from both BUN and creatinine. Although several studies have shown that high BUN/Creatinine ratio is associated with worse outcome in patients with HF, no study showed the prognostic significance of BUN/Creatinine ratio independently from BUN[4-10].

In the present study, we found BUN/Creatinine varied widely even in the general population, probably due to the influence of non-renal factors on both creatinine and BUN. BUN/Creatinine ratio was significantly higher in patients with AHF than normal subjects in line with the current concept; however, BUN/Creatininne ratio was still within the predicted 95% interval in more than 70% of AHF patients. This implies that it is difficult to evaluate BUN/Creatinine ratio just by itself and without any reference. Our study results also showed that BUN/Creatinine ratio was an age- and sex-dependent variable. According to these results, we evaluated BUN/Creatinie ratio based on prediction interval after incorporating these properties. We speculate that this approach makes it possible to identify the AHF patient whose BUN/Creatinine ratio is irrationally high and at high risk of worse prognosis.

Limitations

This study has several important limitations. This study was performed retrospectively, and some subjects with missing BUN/Creatinine data were excluded in both PROTECT and PREVEND datasets. Additional factors that can affect BUN/Creatinine ratio including corticosteroid and some antibiotics use were not assessed. Both datasets primarily consist of Caucasian subjects and our study results might not be applicable to other ethnicities. We used the PREVEND dataset after excluding patients with cardiovascular comorbidity to derive general cohort and to estimate BUN/Creatinine from age and sex; however, this may not be sufficient to determine normal range of BUN/Creatinine ratio. By design, PREVEND dataset was enriched for subject with increased albumin excretion in urine. We performed a weighted analysis to correct this; however, there was a possibility that study design affected our results In the PROTECT study, only AHF patients with mild renal dysfunction were included and external validation for a wider cohort of AHF patients is not included in the present analysis. Only up to 180 day mortality and 60 days rehospitalization data were available by study design and impact of higher than normal range of

BUN/Creatinine ratio on long-term prognosis remains to be investigated. As this is an AHF cohort, very few patients were classified as lower than normal range of BUN/Creatinine ratio and our analysis did not have enough power to evaluate prognostic significance of this group.

Conclusions

BUN/Creatinine ratio was an age- and sex-related variable, and it varied widely in the general population. BUN/Creatinine ratio was higher in patients with AHF compared to the general population, and a quarter of AHF patients had higher than normal range of BUN/Creatinine ratio. In AHF patients, higher than normal values of BUN/Creatinine ratio were associated with worse outcome and provided additive prognostic information on top of pre-existing prognostic factors including creatinine and BUN.

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Supplemental materials

Supplemental Table 1. Study subjects excluded from or included in the analysis with general population (PREVEND)

Factors	Included (N=4484)	Excluded (N=3490)	P value
Age	44±11	56±11	<0.001
Male gender	2012 (45)	1956 (56)	<0.001
Race (%)			
Caucasian	4226 (95)	3331 (96)	0.006
Negroid	48 (1)	31 (1)	
Asian	109 (2)	57 (2)	
Other	72 (2)	35 (1)	
BMI	25±4	28±4	<0.001
Systolic blood pressure (mmHg)	118±11	142±21	<0.001
Diastolic blood pressure (mmHg)	70±7	80±10	<0.001
Heart rate (bpm)	68±10	70±11	<0.001
Smoking history (within 1year)	1760 (39)	1250 (36)	0.002
Stroke history	19 (0.4)	54 (1.6)	<0.001
Creatinine (mg/dl)	0.90 (0.81-1.01)	0.96 (0.85-1.07)	<0.001
BUN (mg/dl)	14 (12-16)	15 (13-18)	<0.001
Cholesterol (mg/dl)	199 (178-220)	244 (213-271)	<0.001
Glucose (mg/dL)	83 (76-88)	88 (81-99)	<0.001
hs-CRP (mg/L)	0.9 (0.4-2.3)	1.8 (0.9-3.9)	<0.001
NT-proBNP (pg/mL)	32.9 (15.2-60.9)	46.0 (20.0-98.7)	<0.001
UAE (mg/24h)	8.0 (5.8-12.6)	12.5 (7.4-29.6)	<0.001
BUN/Creatinine ratio	15.0 (13.0-17.6)	15.8 (13.4-18.7)	<0.001

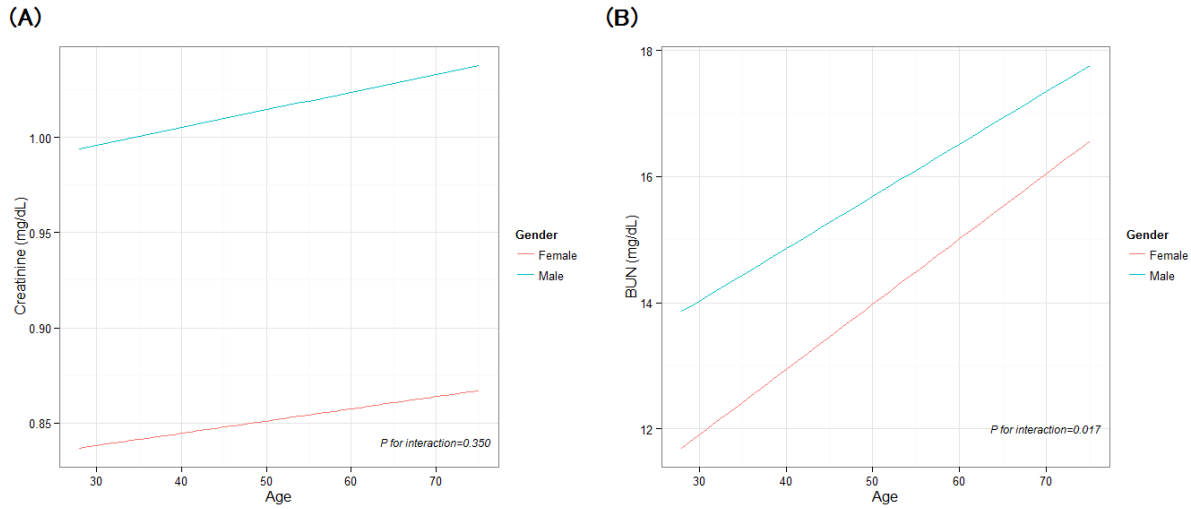
BMI, body mass index; BUN, blood urea nitrogen; BUN/Creatinine, blood urea nitrogen to creatinine; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; UAE, urinary albumin excretion

Supplemental Table 2. Cox regression analysis for outcomes in acute heart failure patients age 75 or below (PROTECT)

Outcomes	Univariate Cox			Adjusted for clinical model*		
	HR	95%CI	P value	HR	95%CI	P value
Death through Day 180						
BUN/Creatinine ratio within normal range	1 (Reference)			1 (Reference)		
BUN/Creatinine ratio lower than normal range	-	-	-	-	-	-
BUN/Creatinine ratio higher than normal range	2.77	2.06-3.71	<0.001	2.09	1.27-3.45	0.003
Death or Cardiovascular or Renal Rehospitalization through Day 60						
BUN/Creatinine ratio within normal range	1 (Reference)			1 (Reference)		
BUN/Creatinine ratio lower than normal range	0.65	0.21-2.04	0.462	0.84	0.25-2.82	0.78
BUN/Creatinine ratio higher than normal range	1.86	1.50-2.32	<0.001	1.35	0.94-1.93	0.100
HF Rehospitalization through Day 60						
BUN/Creatinine ratio within normal range	1 (Reference)			1 (Reference)		
BUN/Creatinine ratio lower than normal range	0.81	0.20-3.29	0.770	1.17	0.26-5.27	0.837
BUN/Creatinine ratio higher than normal range	1.54	1.13-2.09	0.006	1.06	0.64-.176	0.830

*Adjusted for age, previous heart failure hospitalization, peripheral edema, systolic blood pressure, sodium, log blood urea nitrogen, log creatinine and albumin

Supplemental Figure 1. Association between (A) age and creatinine, and (B) age and BUN in general population by sex (PREVEND)



Chapter 4

Clinical effectiveness of tolvaptan in patients with acute decompensated heart failure and renal failure: design and rationale of the AQUAMARINE study

Yuya Matsue
Makoto Suzuki
Wataru Nagahori
Kazuki Yoshida
Yuko Onishi
Yasuhiro Satoh
Yuichi Ono
Toshihiko Nishioka
Makoto Noda
Kaoru Sugi
Sho Torii
Tamotsu Tejima
Harumizu Sakurada
Satoshi Yamaguchi
Kaoru Okishige
Hiroyuki Fujii
Atsushi Takahashi

Cardiovasc Drugs Ther 2014;28:73-77

Abstract

Purpose

Over half of all admitted acute decompensated heart failure (ADHF) patients have renal failure. Although diuretics represent the mainstay of treatment strategy even in this population, there are unmet needs for safer and more effective treatment. Tolvaptan is a vasopressin-2 receptor antagonist, and we hypothesized that adding tolvaptan to standard diuretic therapy would be more effective in ADHF patients with renal function impairment.

Methods

The Answering question on tolvaptan's efficacy for patients with acute decompensated heart failure and renal failure (AQUAMARINE) is a multicenter, randomized controlled clinical trial, which will enroll 220 patients from 17 hospitals in Japan. ADHF patients whose estimated glomerular filtration rate is above 15 and below 60 mL/min/1.72 m^2 will randomly assign within 6 h after admission to usual care with furosemide or tolvaptan add-on therapy. Primary endpoint is achieved urine output within 48 hours. Secondary endpoints include dyspnea relief measured by 7-points Likert scale, incidence of worsening renal function, dose of furosemide used within 48 h, and changes of brain natriuretic peptide.

Conclusion

This study is the first multicenter study in Japan to evaluate clinical effectiveness of tolvaptan add-on therapy in ADHF patients with renal failure. Results of this study address treatment strategy of this high-risk population (UMIN Clinical Trial Registry Number: UMIN000007109).

Key words: Diuretics; Acute decompensated heart failure; Vasopressin antagonist; Cardiorenal.

Introduction

Acute decompensated heart failure (ADHF) is one of the most common causes of hospitalization and death in patients with cardiovascular disease. The rate of admission due to ADHF has more than doubled in the past 30 years in United States [1]. According to the Japanese Nationwide Registry of Acute Heart Failure (ATTEND registry), the in-hospital mortality rate is 6.4% and median duration of admission is 21 days in Japan [2]. The main problem of ADHF is fluid retention, clinically expressed by systemic and pulmonary congestion, and so intravenous loop diuretics have been the mainstay form of treatment for ADHF despite a lack of robust clinical evidence.

Renal function deterioration in ADHF, which is commonly described as "Cardiorenal Syndrome Type 1," is very common in daily practice [3]. Among patients included in the ADHERE registry, the estimated glomerular filtration rate (eGFR) at admission was below 60 mL/min/1.73 m^2 in 63.6% of all ADHF patients. The mean serum creatinine clearance in Japanese patients was 1.43 mg/dL in the ATTEND registry [2]. As the mean age of registered patients was reported as 73.0 years old, we estimated that over half of all patients have renal dysfunction at admission due to ADHF even in Japan.

One of the clinical issues of this renal impairment is a reduced response to diuretics (i.e., diuretic resistance). This phenomenon is multifactorial and poorly understood, and therefore there is no proven treatment strategy for this population. Diuretic refractoriness is influenced mostly by renal function and low-dose diuretic administration [4, 5]. Therefore, we commonly up-titrate the dose of diuretics in patients with impaired renal function. However, this direction of therapy may fall into a vicious cycle: high-dose diuretic use is associated with activation of the renin–angiotensin–aldosterone system and sympathetic nervous system, both of which lead to reduce renal blood flow [6, 7].

Recently, several non-peptide vasopressin receptor antagonists have entered clinical development, including tolvaptan. Tolvaptan is an oral, once-daily, vasopressin-2 receptor antagonist. In contrast to furosemide, tolvaptan has been shown to achieve urine output without decreasing renal blood flow in heart failure patients [8]. Moreover, tolvaptan seems not to activate the sympathetic nervous system and the renin–angiotensin–aldosterone system [9].

The Answering question on tolvaptan's efficacy for patients with acute decompensated heart failure and renal failure (AQUAMARINE) is a randomized control trial to evaluate the hypothesis that we can treat patients with ADHF and renal failure more effectively and safely by adding tolvaptan to standard diuretic therapy compared to conventional treatment strategies.

Methods

Study Design

AQUAMARINE is a multicenter, prospective, randomized, controlled trial to compare conventional therapy with tolvaptan add-on therapy in ADHF patients. This study is being performed at 17 hospitals, consisting of 3 university hospitals, 11 public hospitals, and 3 private hospitals, in 5 prefectures in Japan. All study activities are being

coordinated by the Data Coordinating Center at Kameda Medical Center in Kamogawa, Chiba, Japan.

Objectives

The primary objective of AQUAMARINE is to determine the clinical effectiveness of tolvaptan add-on therapy in patients with ADHF and renal failure, which is defined as eGFR below 60 mL/min/1.73 m^2, compared to conventional therapy. The secondary objectives are to evaluate the clinical safety of using tolvaptan in ADHF and renal failure patients.

Patient population, inclusion and exclusion criteria

This study received approval from the institutional review board/ethics committee at each site and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent is obtained from all patients before including and randomizing. Only patients admitted to hospital with primary diagnosis of ADHF are considered eligible for enrollment. We include only patients with renal dysfunction at the point of admission (defined as eGFR below 60 mL/min/1.73 m^2 on the day of inclusion) and apparent signs of congestion: jugular venous distention, pitting edema, or dyspnea. eGFR is calculated by using Modification of Diet in Renal Disease Study equation coefficients modified for Japanese [10]. Inclusion and exclusion criteria are listed in **Table 1**.

Table 1. Inclusion and Exclusion criteria

Inclusion and Exclusion Criteria
Inclusion Criteria
1. 20 ≤Age< 85 years old
2. Admitted to hospital with a primary diagnosis of ADHF
3. Have at least one sign of congestion (peripheral edema, pulmonary congestion, pleural effusion, jugular venous distention, orthopnea)
4. 15 ≤ eGFR ≤ 60 mL/min/1.73 m^2
Exclusion Criteria
1. Requiring mechanical circulatory support
2. Consciousness disturbance
3. Hypernatremia (serum Na at admission > 147 mEq/l)
4. Volume depletion
5. Cardiac shock
6. Allergy or contraindication for tolvaptan
7. Acute coronary syndrome
8. Chronic hemodialysis

ADHF, acute decompensated heart failure; eGFR, estimated glomerular filtration rate

Randomization and intervention

Eligible patients who agree to participate in the study are randomized in a one-by-one fashion using a web-based randomization system to receive tolvaptan add-on therapy (15 mg once daily for 2 days in addition to conventional therapy) or conventional therapy only within 6 h of admission (**Figure 1**). Increasing or decreasing the dose of tolvaptan in the study period (2 days) is not allowed. After the study period, the clinical decision regarding whether to continue or discontinue tolvaptan is left to each attending physician. In conventional therapy for ADHF, whether to use standard heart failure drugs, including angiotensin-converting-enzyme inhibitors, angiotensin receptor blocker, vasodilators, beta-blockers, and digoxin, is left to the discretion of the attending physician.

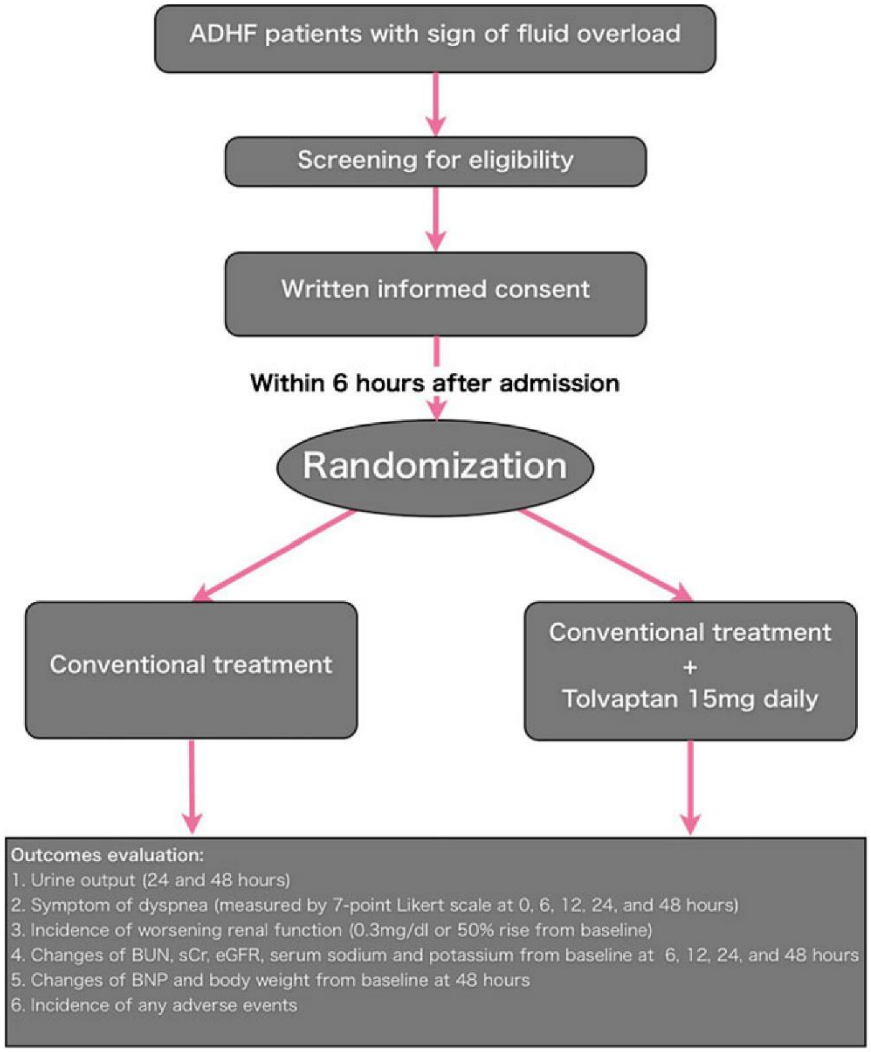


Fig 1. Study protocol of AQUAMARINE

Endpoints

The primary endpoint is urine output within 48 h after randomization. Secondary endpoints include incidence of worsening of renal function (0.3 mg/dl or 50% serum creatinine rise from baseline within 48 h) and dose of furosemide used within 48 h after randomization. Patients' self-reported symptoms (seven-point Likert scale of change compared with baseline) are also measured and recorded at 6, 12, 24, and 48 h after randomization, and dyspnea relief is also compared between the two groups. Changes in blood urea nitrogen, serum creatinine, eGFR, brain natriuretic peptide, serum sodium, serum potassium, signs of congestion, and body weight are also measured as secondary endpoints. Serum creatinine, blood urea nitrogen, sodium, and potassium are measured at baseline and 6, 12, 24, and 48 h after randomization. Brain natriuretic peptide and body weight are measured at baseline and 48 h after randomization. Any adverse outcome, including induction of mechanical ventilation, and requirement of any renal replacement therapy are also included as secondary endpoints.

Statistical analysis

According to previous observational research, the standard deviation of urine output within 48 h after admission is 2350 ml [11]. Therefore, we decided a sample size of 110 patients per treatment arm to provide 85% power for detecting a difference between treatment groups in urine output within 48 h of 1000 ml after making allowance for 8% dropout after randomization.

The primary endpoint will be analyzed by the two-sample t-test. The secondary endpoints will be analyzed by the two-sample t-test (continuous outcome) and the chi-squared test (categorical outcomes).

Discussion

AQUAMARINE is designed to evaluate the clinical effectiveness of tolvaptan add-on therapy for patients with ADHF and renal failure. Treatment of this cohort is very challenging not only due to diuretic resistance, but also the greater length of hospital stay, readmission, and high in-hospital mortality rate have been reported in previous studies [12-14]. However, as a considerable proportion of ADHF patients have renal failure at the time of admission, it is necessary to search for a better treatment strategy. In some cases, intravenous diuretics for this population may lead to further worsening of renal function throughout direct and indirect adverse effects on the kidney. There is, however, a dilemma that ADHF patients require decongestion as soon as possible, and diuretics are prerequisites for decongestion in ADHF patients even in renal failure. Tolvaptan has emerged as a new-class of diuretic differing from furosemide in its favorable effects on renal blood flow, neurohormones, and blood pressure [8, 9, 15, 16]. These factors play crucial roles in renal function even in heart failure. Therefore, we hypothesize that tolvaptan would be especially effective in ADHF and renal failure patients.

The effects of tolvaptan in ADHF patients were evaluated in a double-blind randomized clinical trial (EVEREST). In this study, tolvaptan showed significant weight reduction and improvement of dyspnea compared to placebo in the

short-term, but no prognostic benefit in medium-term outcome [17, 18]. However, there were some differences in study design between EVEREST and AQUAMARINE: dose and duration of tolvaptan use (30 mg and minimum of 60 days vs. 15 mg and 2 days), time to randomization (within 48 h vs. 6 h), and patients' renal function. Time to randomization in clinical trials regarding ADHF is of primary importance. To date, clinical trials concerning ADHF, including EVEREST, enrolled patients relatively late (24 – 48 h after admission) in the acute phase [17, 19, 20]. However, dyspnea was improved in 76% of ADHF patients within 6 h [21]. This suggests that the adequate time window for enrolling patients in clinical trials to evaluate the effectiveness of treatments in ADHF is the very early phase [22], and this is why we decided a time window of within 6 h for enrollment in our trial. The dose of tolvaptan used in our study (15mg) is lower than that commonly used in US (30mg). However, in phase II study which was conducted in Japanese HF patients, all three dose (15mg, 30mg, and 45mg) decreased body weight and improve the signs of volume overload, and these effects were not dose-dependent [23]. This is the rationale behind the choice of 15mg dose in this study.

In conclusion, AQUAMARINE is a multicenter, open-label, randomized trial for evaluating the clinical effectiveness of tolvaptan as additive therapy in the acute phase of ADHF in patients with renal failure. The results of this study will be beneficial for determining the appropriate treatment strategies for ADHF patients.

Acknowledgments

AQUAMARINE is supported by a Multicenter Clinical Research Grant from the Japan Heart Foundation. Clinical trial registration ID assigned by the University hospital Medical Information Network (UMIN) as UMIN000007109.

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Cardiovasc Drugs Ther. 2011; 23(suppl 1): S19-S31.

Chapter 5

Clinical Effectiveness of Tolvaptan in Patients with Acute Heart Failure and Renal Dysfunction - AQUAMARINE Study –

Yuya Matsue
Makoto Suzuki
Sho Torii
Satoshi Yamaguchi
Seiji Fukamizu
Yuichi Ono
Hiroyuki Fujii
Takeshi Kitai
Toshihiko Nishioka
Kaoru Sugi
Yuko Onishi
Makoto Noda
Nobuyuki Kagiyama
Yasuhiro Satoh
Kazuki Yoshida
Steven R. Goldsmith

J Card Fail 2016;22:423-432

Abstract

Background

More efficacious and/or safer decongestive therapy is clearly needed in acute heart failure (AHF) patients complicated by renal dysfunction. We tested the hypothesis that adding tolvaptan, an oral vasopressin-2 receptor antagonist, to conventional therapy with loop diuretics would be more effective treatment in this population.

Methods and Results

A multicenter, open-label, randomized control trial was performed, and 217 AHF patients with renal dysfunction (estimated glomerular filtration rate 15 – 60 mL/min/1.73 m2) were randomized 1:1 to treatment with tolvaptan (n = 108) or conventional treatment (n = 109). The primary endpoint was 48-hour urine volume. The tolvaptan group showed more diuresis than the conventional treatment group (6464.4 vs. 4999.2 mL, $P < 0.001$) despite significantly lower amounts of loop diuretic use (80 mg vs. 120 mg, $P < 0.001$). Dyspnea relief was achieved significantly more frequently in the tolvaptan group at all time points within 48 hours except 6 hours from enrollment. The rate of worsening of renal function (≥ 0.3 mg/dL increase from baseline) was comparable between tolvaptan and conventional groups (24.1% vs. 27.8%, respectively; $P = 0.642$).

Conclusions

Adding tolvaptan to conventional treatment achieved more diuresis and relieved dyspnea symptoms in AHF patients with renal dysfunction.

Clinical Trial Registration

URL: http://www.umin.ac.jp/ctr/index/htm/ Unique identifier: UMIN000007109

Key words: Vasopressin antagonist, acute heart failure, renal function

Introduction

Acute heart failure (AHF) is one of the most important causes of hospitalization worldwide, and the prognosis of this disease remains unsatisfactory[1-3]. Renal dysfunction, which is usually defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m2, is highly prevalent in AHF patients[4, 5]. Treatment of AHF patients complicated with renal dysfunction is still challenging because this population may be relatively refractory to treatment with loop diuretics, which may in themselves aggravate renal dysfunction. Patients with AHF and renal dysfunction have prolonged hospital stays, high in-hospital and long-term mortality rates[6, 7]. No therapy thus far tested has improved upon either short-term or long-term outcomes in these patients, including most recently ultrafiltration, standard and low-dose nesiritide, and low-dose dopamine[8, 9]. To date, therefore, there is no therapy yet proven to be either more efficacious or safe in this patient population when compared to loop diuretics. Given the high prevalence of renal dysfunction and the poor outcomes in these patients, there remains an unmet need to develop a new treatment strategy.

Tolvaptan is an oral, nonpeptide, selective vasopressin-2 receptor antagonist that acts on the distal portion of the nephron, blocking the interaction of the antidiuretic hormone arginine vasopressin and the V2 receptor. This prevents the activation of the aquaporin system, impairs the ability of the kidney to reabsorb water, and as a result causes an increase in free water excretion. Tolvaptan has been shown to be safe and effective in the correction of euvolemic and hypervolemic hyponatremia including that seen in patients with heart failure[10]. The efficacy of tolvaptan as a treatment for AHF was evaluated in the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan (EVEREST) trial[11, 12]. However, the EVEREST study did not specifically target AHF patients complicated with renal dysfunction. In other studies, tolvaptan showed beneficial effects on renal function, such as maintenance of renal blood flow and no activation of the sympathetic nervous and renin-angiotensin-aldosterone systems compared to loop diuretics[13]. In a previous small retrospective study, we reported that addition of tolvaptan to conventional therapy yielded more urine with less furosemide use and less worsening of renal function (WRF)[14]. We therefore designed the AQUAMARINE study to evaluate the effects of tolvaptan on decongestion, clinical signs and symptoms, and renal function when added to conventional therapy in patients with AHF and diminished renal function defined as an eGFR less than 60 mL/min/1,73m2.

Methods

Study design

The AQUAMARINE study was a prospective, multicenter, randomized, open-label, parallel group study to evaluate the short-term efficacy of adding tolvaptan to conventional therapy for treatment of AHF. The study background and design have been published previously[15]. Briefly, AHF patients admitted to hospital and complicated with renal dysfunction (eGFR 15 – 60 mL/min/1.73 m2 by simplified Modification of Diet in Renal Disease estimated glomerular filtration rate equation for Japanese16) at the time of admission were enrolled in the study within 6

hours from admission at 14 hospitals across Japan. The diagnosis of heart failure was based on the Framingham criteria, but at least one of the following five signs or symptoms of congestion was required: peripheral edema, pulmonary congestion, pleural effusion, jugular venous distension, or orthopnea. Key exclusion criteria included diagnosis of acute coronary syndrome on admission, hypernatremia defined by serum sodium > 147 mEq/L, and chronic hemodialysis.

All enrolled patients were randomized 1:1 in an open fashion to receive conventional therapy or tolvaptan add-on therapy by an automated web-based randomization system created by a third-party company (Mebix, Inc., Tokyo, Japan). We applied a minimization procedure when randomizing using eGFR < 30 mL/min/1.73 m2, blood pressure > 140 mmHg, and age > 60 years old as stratification factors. The patients allocated to the tolvaptan group received oral tolvaptan (15 mg/day) for 2 days; the day of enrollment and the following day. In addition, physicians were permitted to treat the patients with any conventional therapy at their discretion. Fluid restriction was also permitted at the physician's discretion during study period. The choice of conventional therapy for AHF, i.e. standard heart failure drugs, including angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers, vasodilators, beta-blockers or digoxin, is left to the attending physician. After 2 days of mandatory tolvaptan treatment in the tolvaptan group, physicians determined whether to continue tolvaptan or not according to each patient's status. Similarly, treatment with tolvaptan was prohibited for the first 2 days in the patients allocated to the conventional group. The investigation conforms with the principles outlined in the Declaration of Helsinki. The ethics committee at each center approved the study, and all patients provided written informed consent.

Endpoints

The primary endpoint was amount of urine output within 48 hours of randomization. The secondary endpoints were: (1) incidence of WRF defined as an increase in serum creatinine (≥0.3 mg/dL increase from baseline) at various pre-specified time points (6, 12, 24, and 48 hours from randomization); (2) moderate or marked improvement of dyspnea from baseline according to patient-reported 7-point Likert scale measured at 6, 12, 24, and 48 hours from enrollment; (3) amount of furosemide-equivalent loop diuretics used within 48 hours; (4) changes in blood pressure, heart rate, serum sodium, serum potassium, serum creatinine, eGFR, and blood urea nitrogen at 6, 12, 24, and 48 hours from enrollment; (5) changes in brain natriuretic peptide (BNP) and body weight from baseline to 48 hours; (6) incidence of any adverse events; and (7) combined endpoint of all cause death and heart failure rehospitalization within 90 days.

Statistical analysis

A sample size of 110 patients for each group was decided based on our previous study data to detect a difference of 1000 mL in urine output within 48 hours between the two groups with 85% power and 5% alpha-error, while allowing for an 8% drop-out rate[14].

Pre-specified subgroup analyses were conducted for patients with age ≥ 75 years old, heart failure with preserved ejection fraction (HFpEF) (≥ 50%), non-ischemic etiology, systolic blood pressure (SBP) > 140 mmHg, eGFR < 30 mL/min/1.73 m2, atrial fibrillation at admission, above the median of blood urea nitrogen (BUN), and above the

median of BUN/creatinine ratio.

The primary outcome as well as other two-group comparisons of continuous outcomes were performed with Student's t test for independent variables and paired t test for paired variables. For non-normally distributed continuous outcomes, Wilcoxon's rank sum test was used for independent variables and Wilcoxon's signed rank test for paired variables. Categorical outcomes were tested with Pearson's chi-squared test or Fisher's exact test. For repeatedly measured outcomes, linear mixed effect models were used to examine the interactions between these variables and time to test differences in trajectories over time. All treatment comparisons were performed according to the intention-to-treat principle. Two-tailed $P < 0.05$ was taken to indicate statistical significance for all analyses. All statistical analyses were conducted with R version 3.1.3.

Results

Patient background

A total of 220 patients were enrolled between December 2011 and January 2015. After randomization, one patient in the tolvaptan group and one patient in the conventional group withdrew their consent and data were missing for one patient in the tolvaptan group. Therefore, after excluding these three patients, 217 patients were finally included in the final analysis (Figure 1). The baseline characteristics of the patients were similar between both groups (Table 1). The median age of the patients was 75 years (interquartile range [IQR], 68 – 81 years), and 64.9% were male. The median left ventricular ejection fraction was 44.5%, and 82 (37.8%) of the whole cohort had left ventricular ejection fraction ≥ 50%. The reported etiologies of participants were as follows: ischemic etiology, 60 (27.6%); cardiomyopathy, 69 (31.8%); hypertensive AHF, 50 (23.0%); valvular disease, 32 (14.7%); and others, 25 (11.5%) patients. The mean eGFR at baseline was 40.5 mL/min/1.73 m2, and 57 (26.3%) patients had eGFR < 30 mL/min/1.73 m2. The median time from admission to randomization was 1 hour, and 41.4% of patients were randomized before admission at either emergency department or clinic.

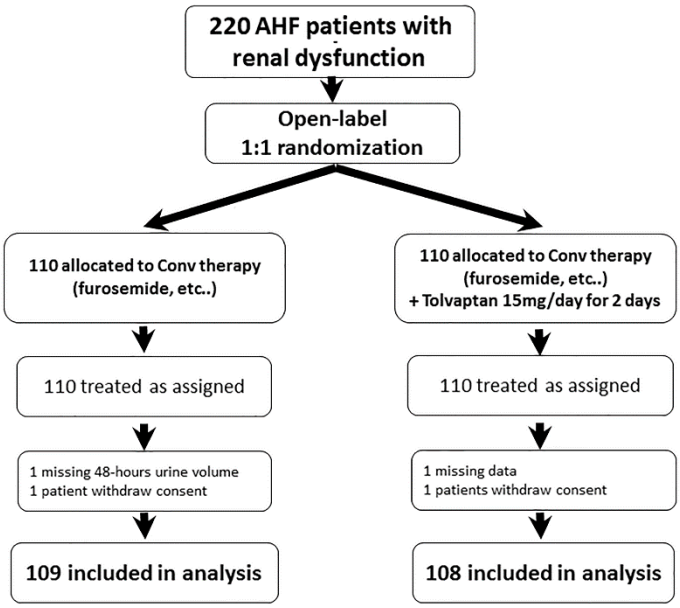

Figure 1. Study flow chart including number of patients who underwent assignment.
AHF, acute heart failure

Table 1. Patient characteristics

Variables	Conventional Group (*n* = 109)	Tolvaptan Group (*n* = 108)
Age (years)	72.95 ± 10.24	72.99 ± 8.90
Male (%)	69 (63.3)	72 (66.7)
Systolic blood pressure (mmHg)	142.1 ± 28.1	145.8 ± 32.9
Diastolic blood pressure (mmHg)	82.4 ± 20.2	83.2 ± 24.0
Heart rate (bpm)	88.6 ± 23.4	94.2 ± 27.3
LVEF (%)	46.8 ± 16.4	45.4 ± 18.1
HFpEF (%)	42 (39.3)	40 (37.4)
NYHA III/IV (%)	69 (63.3)	79 (73.1)
Medical History (%)		
HF admission	47 (43.1)	48 (44.4)
Hypertension	83 (76.1)	82 (76.6)
Diabetes	54 (49.5)	42 (38.9)
Dyslipidemia	47 (43.1)	45 (41.7)
Atrial fibrillation	55 (50.5)	60 (55.6)
Smoking (Current or Ex)	54 (51.4)	57 (53.8)
Drugs at admission (%)		
Loop diuretics	47 (43.1)	43 (39.8)

Furosemide equivalent loop diuretic dose among users (mg)	40.0 [20.0 – 60.0]	30.0 [20.0 – 45.0]
ACE-I/ARB	41 (37.6)	45 (41.7)
Beta blocker	43 (39.4)	41 (38.0)
Aldosterone antagonist	27 (24.8)	19 (17.6)
Digoxin	5 (4.6)	7 (6.5)
Furosemide equivalent amount of loop diuretics used before randomization (mg)	20.0 [20.0 – 20.0]	20.0 [20.0 – 20.0]
Time to Randomization (hours)	1.0 [0.0 – 2.0]	1.0 [0.0 – 3.0]
IV therapy within 48 hours (%)		
Carperitide	39 (35.8)	41 (38.0)
Nitrate/ISDN	20 (18.3)	24 (22.2)
Nicorandil	1 (0.9)	2 (1.9)
Heparin	49 (45.0)	43 (39.8)
Dopamine	1 (0.9)	2 (1.9)
Dobutamine	16 (14.7)	8 (7.4)
PDEIII inhibitor	0 (0.0)	1 (0.9)
Lab data		
Creatinine (mg/dL)	1.4 ± 0.5	1.5 ± 0.6
eGFR (mL/min/1.73 m^2)	41.4 ± 13.4	39.5 ± 12.8
BUN (mg/dL)	25.0 [18.9 – 35.0]	28.0 [20.0 – 37.1]
Serum sodium (mEq/L)	140.2 ± 3.8	140.5 ± 4.3
Serum potassium (mEq/L)	4.3 ± 0.7	4.4 ± 0.6
BNP (pg/mL)	729.0 [461.9 – 1482.2]	939.3 [532.9 – 1510.8]

LVEF, left ventricular ejection fraction; HFpEF, heart failure with preserved ejection fraction; NYHA, New York Heart Association functional class; HF, heart failure; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; PDE, phosphodiesterase; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; BNP, brain natriuretic peptide.

All patient were treated according to allocation. However, four patients took tolvaptan on only 1 day and it was discontinued thereafter. The reasons for discontinuation of tolvaptan were hypernatremia in three patients and septic shock in one patient. The median duration of tolvaptan treatment in the tolvaptan group was 2 days (IQR: 2 – 4 days). Continuation, discontinuation, and new prescription of angiotensin converting enzyme inhibitor, angiotensin receptor blocker, and aldosterone antagonist during study period (after randomization) is shown in

Supplemental Table 1. There was no significant difference between groups.

Primary endpoint

Tolvaptan therapy yielded significantly higher 48-hour urine output compared to conventional therapy (conventional group, 4997.2 mL; 95% CI, 4598.3 – 5400.0 mL vs. tolvaptan, 6464.4 mL; 95% CI, 5859.1 – 7069.7 mL; P < 0.001), and the mean difference between the two groups was 1465 mL (95% CI: 740.7 – 2189.7 mL) within 48 hours (Table 2).

Table 2. Summary of primary and secondary endpoints

Outcomes	**Conventional group (*n* = 109)**	**Tolvaptan group (*n* = 108)**	***P*-value**
Primary outcome			
48-hour urine volume (mL)	4997.2 ± 2101.4	6464.4 ± 3173.0	< 0.001
Secondary outcomes			
Worsening of renal function (%)	30 (27.8)	26 (24.1)	0.642
Dose of diuretics use within 48 hours (mg)	120 (80 – 180)	80 (40 – 150)	< 0.001
Net fluid loss within 48 hours (mL)	3697.9 ± 2112.0	4700.1 ± 2443.3	0.004
Change in BNP from baseline to 48 hours (pg/mL)	-306.1 (-153.7 to -662.1)	-285.3 (-110.7 to -650.9)	0.602
Change in body weight from baseline to 48 hours (kg)	-1.99 ± 2.17	-3.16 ± 2.66	< 0.001
Length of hospital stay (day)	14.6 (10.3 – 27.2)	14.2 (8.9 – 20.3)	0.36
Adverse events (%)	6 (5.5)	10 (9.3)	0.313
In-hospital death (%)	5 (4.6)	4 (3.7)	> 0.99

BNP, brain natriuretic peptide

Secondary Endpoint

The results regarding secondary endpoints are summarized in Table 2. There were no significant differences between groups in the incidence of WRF, trajectory of serum creatinine, eGFR, or serum BUN (Figure 2). We also evaluated the incidence of WRF in subgroup of HFpEF and heart failure with reduced ejection fraction (HFrEF) patients because aggressive fluid removal might lead to subsequent WRF especially in HFpEF[17]. However, there was no difference in incidence of WRF in both HFrEF (26.6% in conventional group vs 23.9% in tolvaptan group, P=0.841) and HFpEF patients (29.5% in conventional group vs 24.4% in tolvaptan group, P=0.633). We also checked the incidence of WRF within 96 hours, which was not a prespecified endpoint, as a sensitivity analysis. There were 5 patients crossover from conventional group to tolvaptan group, and 76 patients (70.3%) were treated without tolvaptan after 48 hours according to physician's decision. WRF within 96 hours was observed in 38 patients (34.9%)

with conventional group and in 31 patients (28.7%) with tolvaptan group, and there was no statistically significant difference (P=0.382).

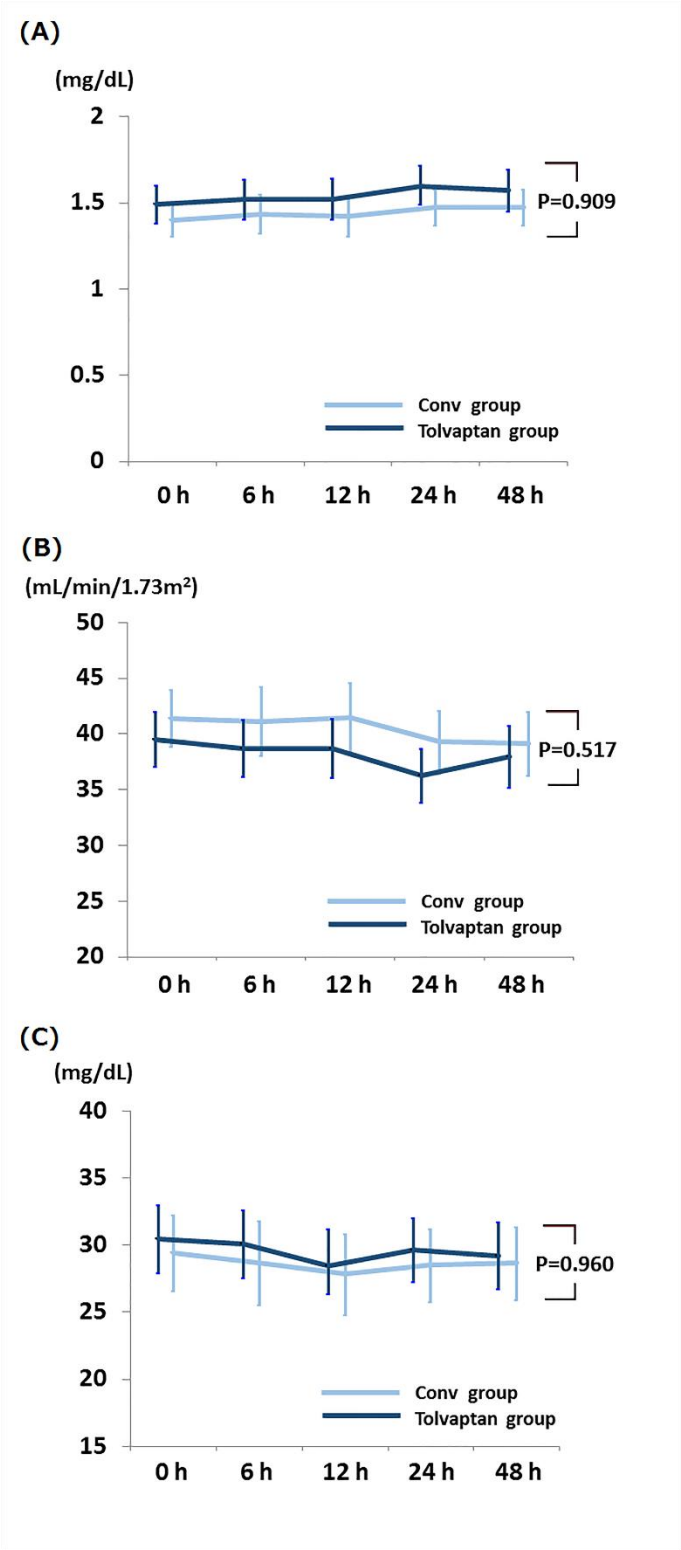

Figure 2. Creatinine (Panel A), eGFR (Panel B), and blood urea nitrogen (Panel C) trajectories from baseline to 48

hours

The amount of furosemide-equivalent diuretics used within 48 hours was significantly lower in the tolvaptan group, and the median difference between the two groups was 40.0 mg (95% CI: 10.0 – 60.0 mg, P = 0.006). Improvement of dyspnea, as defined by moderate or marked improvement from baseline, was significantly more frequent in the tolvaptan group at all time points within 48 hours except 6 hours after randomization (Figure 3 and Supplemental Table 2). Body weight was significantly decreased in both groups from baseline to 48 hours, but the extent of weight loss was significantly greater in the tolvaptan group (mean difference, 1.18 kg; 95% CI, 0.48 – 1.87 kg; P < 0.001). As fluid intake was significantly greater in the tolvaptan group than the conventional treatment group (1319.6 ± 843.3 vs. 1909.3 ± 1300.3 mL, respectively; P < 0.001), net fluid loss was calculated by subtracting total fluid intake within 48 hours from total urine volume within 48 hours and compared between the two groups. The tolvaptan group showed a significantly greater net fluid loss compared to the conventional treatment group (4700.1 ± 2443.3 vs. 3697.9 ± 2112.0 mL, respectively; P = 0.005). BNP was significantly decreased in both groups from baseline to 48 hours (P < 0.001 for both) (Supplemental Table 1), but there was no significant difference in absolute reduction of BNP between the two groups (P = 0.60). Similarly, other signs and symptoms of congestion, including edema, orthopnea, and pulmonary congestion, improved from baseline to 48 hours in both groups (P < 0.001 for all), and there were no significant differences between the groups (Figure 3).

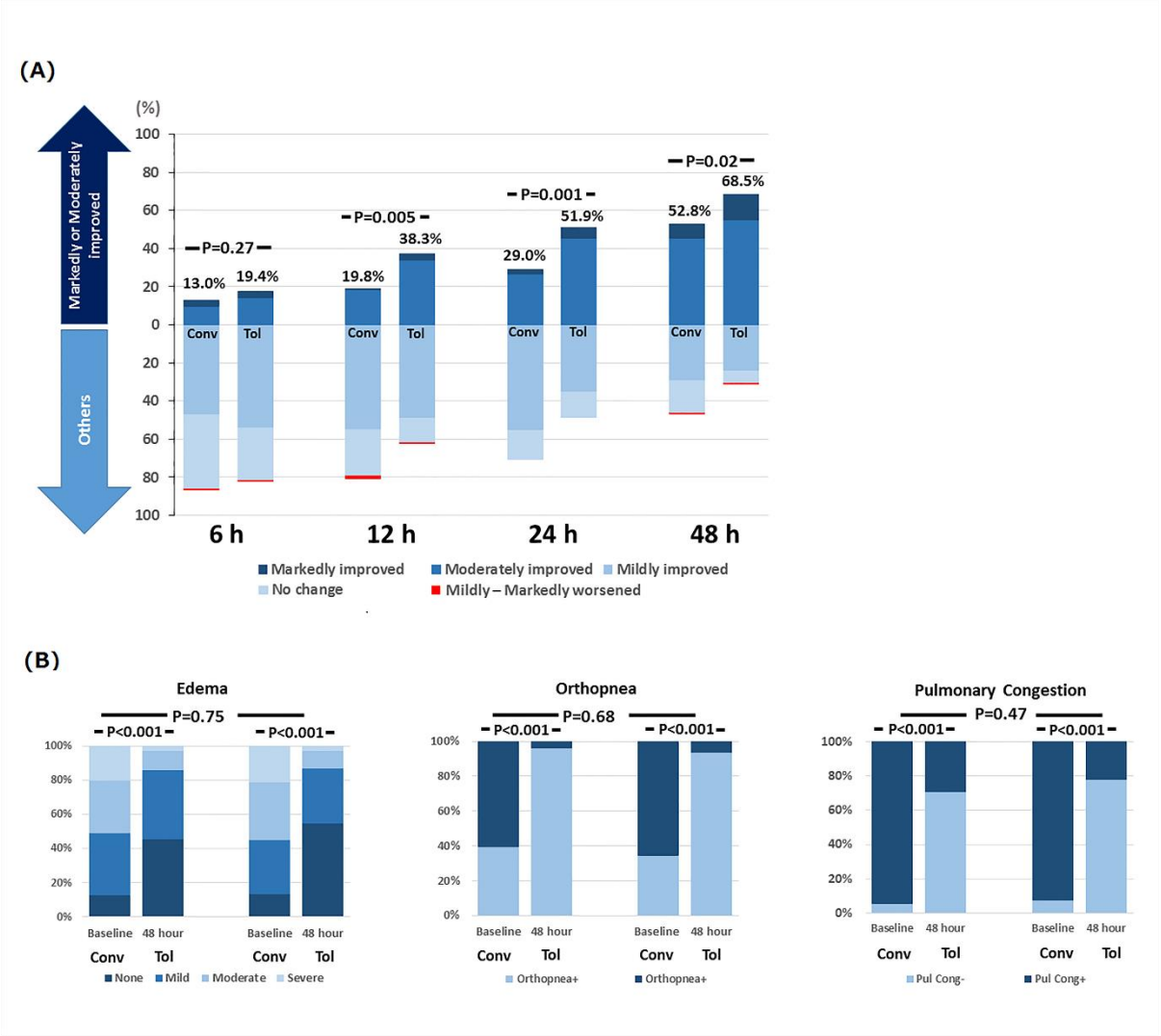

Figure 3. Changes in dyspnea and edematous symptoms within 48 hours. (A) The patients reported changes in dyspnea from baseline measured on a 7-point Likert scale. (B) Changes in congestive symptoms from baseline to 48 hours.
Conv group, conventional group

Heart rate, SBP, and diastolic blood pressure (DBP) decreased from baseline to 48 hours from allocation in both groups (Figure 4). There was no significant difference in heart rate trajectory between groups (P = 0.722). However, the trajectories of both SBP and DBP were significantly different between the tolvaptan and conventional treatment groups (P = 0.005 and P = 0.048, respectively). In addition, we examined the slopes before and after 6 hours by linear splines. There were no significant differences in the initial fall in either SBP or DBP between groups during the first 6 hours. However, both SBP and DBP stabilized in the tolvaptan group (test for a slope of zero; P = 0.997 for SBP and P = 0.776 for DBP), whereas blood pressure continued to fall slightly in the conventional group (test for a slope of zero; P < 0.001 for both SBP and DBP). There were significant between-group differences in time course of changes in both SBP and DBP after 6 hours (test for interaction; P < 0.001 for SBP and P = 0.002 for DBP).

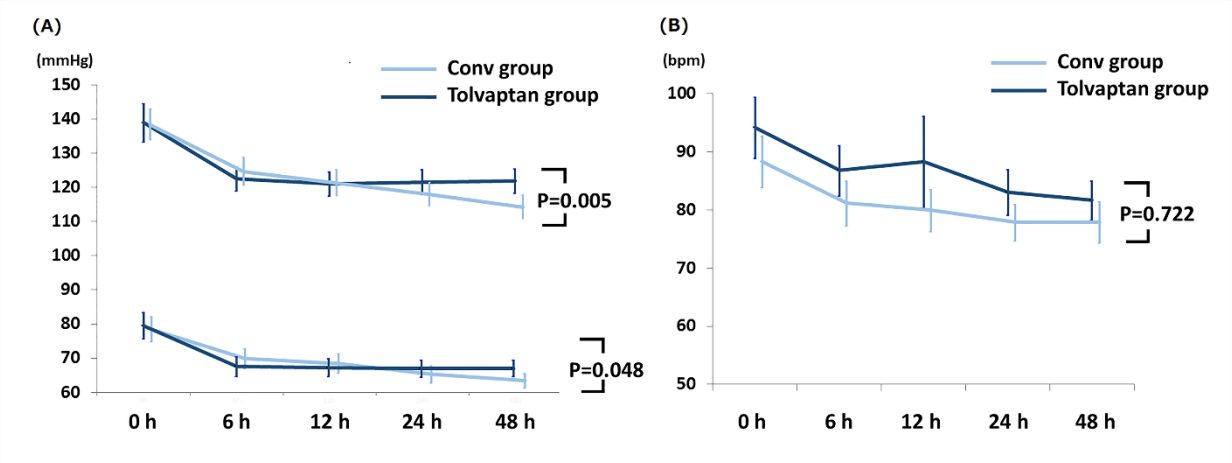

Figure 4. Blood pressure and heart rate changes within 48 hours (A) Graph showing the mean blood pressure at each time point from baseline to 48 hours (B) Graph showing the mean heart rate at each time point from baseline to 48 hours. Error bars indicate 95% confidence intervals
Conv group, conventional group

Similar analyses were performed for repeated measurements of serum sodium and serum potassium to evaluate the differences between the two groups (Supplemental Figure 1). There was a significant difference in overall trajectory of serum sodium between groups ($P < 0.001$). Overall, the potassium and BUN trajectories were not significantly different between groups ($P = 0.382$ and $P = 0.960$, respectively).

We performed pre-specified subgroup analysis and observed consistent results for all subgroups with regard to the primary endpoint (Figure 5). There was no significant difference in median length of hospital stay between groups (conventional group, 14.6 days; IQR, 10.3 – 27.2 days; tolvaptan group, 14.2 days; IQR, 8.9 – 20.3 days; $P = 0.36$). There were no significant differences in the rate of mechanical ventilation or requirement for any renal replacement therapy between groups. Similarly, there were no significant differences in adverse event rate ($P = 0.425$), all cause in-hospital death ($P > 0.99$), or total adverse events and/or all cause in-hospital death ($P = 0.642$). During index hospitalization, five cases of hypernatremia were reported in the tolvaptan group as adverse events; however, none of these cases led to subsequent severe complications and all of these patients recovered spontaneously without any treatment after discontinuing tolvaptan. During follow-up period of 90 days, 28 patients were died or rehospitalized due to heart failure, but there was no statistical significance between groups in this combined outcome (15.0% in conventional group vs. 11.2% in tolvaptan group; P=0.544).

Discussion

In the present study, when compared with conventional care using loop diuretics, the addition of tolvaptan to such care yielded greater net fluid loss and improved dyspnea in AHF patients with moderate to severe renal impairment. Despite greater net fluid loss with tolvaptan, renal function was not worsened. These findings are noteworthy as no other pharmacologic therapy has yielded similar results in this patient population. In the DOSE trial higher doses

of loop diuretics produced more diuresis but also more WRF[18]. In the ASCEND and ROSE trials adding natriuretic peptides and low-dose dopamine, agents expected to improve diuresis and/or improve renal function failed to do so when compared with loop diuretics alone[9, 19]. In the PROTECT trial there was no benefit of an agent specifically designed to offset potential renal dysfunction induced by loop diuretics in this high-risk population[20]. Since renal dysfunction in the setting of AHF confers a poor prognosis, possibly in part due to poor response to diuretics and subsequent unsuccessful treatment, these findings with tolvaptan are of potential importance and suggest the need for larger studies focused on outcomes.

The improvement in net fluid loss, dyspnea relief and preservation of renal function were observed for the entire study group and also in those above the median BUN and below the median eGFR subgroups. Renal function in our cohort was poorer than in other previous AHF trials targeting this population (DOSE, 54.2 mL/min/1.73 m2; ROSE-AHF, 42.7 mL/min/1.73 m2; PROTECT, 53 mL/min/1.73 m2)[9, 18, 20] and so the results in our lowest eGFR group further suggest that tolvaptan may be a promising adjunctive treatment option for AHF patients with moderate to severe renal dysfunction. EVEREST investigators reported positive results with tolvaptan retrospectively in a subgroup with decreased renal function[11, 21], but this was not a prospectively defined subgroup. AQUAMARINE is the first study to study tolvaptan prospectively in this group of patients and it confirms the findings from the retrospective analysis from EVEREST.

We also confirmed the consistent favorable effects of tolvaptan on diuresis in all of our other pre-specified subgroups, including heart failure with preserved ejection fraction. This finding is an important addition to the literature since the only previous large study using tolvaptan in AHF (EVEREST) included only patients with reduced left ventricular ejection fraction (≤ 40%). Takei et al. recently showed that aggressive fluid removal with loop diuretics may be even more problematic for AHF patients with HFpEF compared to HFrEF[17]. This could be explained by the possibility that the aggressive fluid removal caused by conventional diuretic therapy might have caused intravascular volume depletion without refilling from the extravascular space in HFpEF, with subsequent impairment of renal function. In our results, WRF incidence was not increased in patient with HFpEF in spite of more diuresis in tolvaptan group. This is clearly showed utility of tolvaptan in AHF patients with HFpEF, an observation not made in previous trials including EVEREST, and suggests that further trials with this agent should not be limited to patients with HFrEF.

The findings in the current study regarding dyspnea relief with tolvaptan also confirm those seen in the first few days of therapy in the EVEREST and QUEST trials[12, 22]. Although the robustness is limited by our open-label nature, our findings are in line with other double-blinded studies and support usefulness of tolvaptan in this high-risk subgroup given that dyspnea relief is a clinically relevant outcome in AHF patients.

Inconsistent with our previous study[14], however, the incidence of WRF in AQUAMARINE was not reduced by tolvaptan. The incidence of WRF was low in both groups, our predefined observation period was short, and both groups received loop diuretics, all observations that may have made it difficult to demonstrate any beneficial effects

on renal function. The stability of renal function with greater clinical benefit (symptoms and weight loss) could be regarded as a positive rather than a negative finding even at 48 hours, however, since it is possible, that, as seen in DOSE, achieving similar results simply by using more loop diuretics might have increased the incidence of WRF.

The entire issue of the importance of WRF in AHF has grown more complex in the past few years as it has been recognized that WRF is a very heterogeneous phenomenon and that the prognostic implications may be affected by whether or not the WRF is transient and whether or not it is associated with effective decongestion. Indeed, some studies showed that WRF does not adversely affect prognosis in successfully decongested patients[23, 24].

The hemodynamic observations in the present study are also of interest and may relate to the mechanism of action, although without cardiac output and systemic vascular resistance measurements interpretation can only be speculative. However, we did make very close observations of heart rate, SBP and DBP over 48 hours and we found that tolvaptan treatment was associated with better blood pressure maintenance despite greater fluid loss than conventional therapy. This is the first time such an observation has been reported and would be consistent with the effect of tolvaptan to pull fluid from the extravascular space as opposed to repeated direct depletion of the intravascular compartment as one sees with the isosmotic diuresis from furosemide, which might be expected to threaten cardiac preload and so cardiac output in vulnerable patients[25]. Whatever the mechanism this is also a favorable effect of tolvaptan because even modest hypotension is associated with poorer outcome and WRF in AHF[26-28].

Mechanistically, tolvaptan produces diuresis largely by increasing free water diuresis, at least acutely, though over time, an increase in urine sodium has also been observed when the drug is given as monotherapy and compared with loop diuretic[29]. This may be due to the effects of an increase in osmolality attendant upon the free water diuresis. Since water is freely diffusible the early improvement in dyspnea with tolvaptan seen in this and other studies could partially be due to a reduction in lung water, though in the current study we cannot separate this possible effect from that of a greater total diuresis. From the standpoint of renal function and safety, it has been shown that tolvaptan, presumably because of its primary effect as an aquaretic, does not change the sodium concentration at the macula densa, and thereby does not stimulate tubulo-glomerular feedback and/or neurohormonal stimulation, both of which have been linked to worsening renal function with loop diuretics. As already noted, tolvaptan has been shown in one study in human heart failure to have a favorable effect on renal blood flow and GFR as compared to furosemide[13]. These mechanistic observations, in concert with the current data, constitute additional rationale for additional, larger studies with tolvaptan or other V2 antagonists in this high-risk patient group.

However, one note of caution is in order in this regard. A pure V2 antagonist raises osmolality and so stimulates AVP secretion, The V1a effects of AVP may be deleterious in any number of ways both acutely and chronically as the signaling pathways for this receptor resemble those for angiotensin II. Acutely V2 antagonists as noted have been beneficial, but long-term their effects on outcome in heart failure are neutral. Plasma AVP levels were higher in patients on tolvaptan in EVEREST and so the debate continues whether offsetting V1-a stimulation from the increase in plasma AVP might offset the beneficial effects of incremental decongestion from sustained

aquaresis/osmotic diuresis[30]. Conceivably this could occur even in shorter-term studies as well. Therefore, the ideal agent for use in a diuretic-sparing AHF regimen might well be a combined V1a-V2 antagonist but other than one relatively small study demonstrating the safety and efficacy of this approach data are lacking[31], and of course introducing a V1a blocker as part of a strategy to look specifically at non-diuretic decongestive strategies would introduce another level of complexity in the interpretation of any results.

Our study had several limitations, including primarily its open-label nature, which could have influenced some subjective outcome variables, including dyspnea relief, and perhaps more subtly, even objective outcomes if the behavior of treating physicians was affected by knowing that adjunctive therapy was being given in one group. We did not have data on duration of HF prior to being in this study, and it is possible that the diagnosis was relatively new in a substantial number of patients. This might have affected the relatively low usage of guideline recommended medications at baseline. Also we had a relatively large number of patients with HFpEF to whom these guidelines do not apply. As already noted, this was a study focused on short-term responses so the duration of prior therapy would not necessarily have been expected to have a major effect on our results. We did not have sufficient power to detect longer term differences in WRF and prognostic endpoints. Regarding the latter we acknowledge that since this study was conducted in Japan where length of stay is typically much longer than in the US or Europe, any findings related to length of stay or readmission may not be. Relevant to patients treated in these countries. And finally, while this study did include patients with both HPrEF and HFpEF (as have other recent studies in AHF such as DOSE, CARRESS, ROSE, and ASCEND) the average EF was higher and so strictly speaking whether the results could be extrapolated to a patient population more heavily weighted to HFrEF is not known. The similarity of our findings to those in the acute phase of EVEREST is, however, reassuring in this regard.

In conclusion, treating patients with AHF and impaired renal function for 48 hours with tolvaptan in addition to conventional therapy yielded significantly greater net fluid loss and significantly improved dyspnea without worsening renal function. This study when combined with prior trials and mechanistic observations demonstrates the potential utility of using a V2 antagonist as part of a loop-diuretic-sparing strategy as an alternative to the standard approach of simply increasing conventional diuretic therapy in these high-risk AHF patients.

Acknowledgement

I express my deep sense of gratitude to Dr. Tae Ishikawa (Shin-Yurigaoka General Hospital), Dr. Yoshinari Enomoto (Toho University Ohashi Medical Center), Dr. Masakazu Ohno (National Disaster Medical Center), and Dr. Kentaro Toyama (Saitama Medical University) for enrolling study patients and collecting data. I am also thankful to Ms. Noriko Miyazaki for managing this study as a study data and financial manager.

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Supplemental materials

Supplemental Table 1. Medication during the study period

		Conventional Group	Tolvaptan Group	P value
Continuation of the prescribed medication at admission within 48 hours				
	ACE-I/ARB	35/41 (85.4%)	38/45 (84.4%)	>0.99
	Beta blocker	40/43 (93.0)	33/41 (80.5%)	0.113
	Aldosterone blocker	21/27 (80.8)	15/19 (78.9)	>0.99
New prescription within 48 hours				
	ACE-I/ARB	12/68 (17.6)	12/63 (19.0)	>0.99
	Beta blocker	5/66 (7.6)	10/67 (14.9)	0.273
	Aldosterone blocker	10/82 (12.2)	5/89 (5.6)	0.177

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker

Supplemental Table 2. Body weight and brain natriuretic peptide levels at baseline and 48 hours

Variable	Conventional group (n = 109)			Tolvaptan group (n = 108)			P-value for changes from baseline to 48 hours (between groups)
	Baseline	48 hours	P-value (Baseline vs. 48 hours)	Baseline	48 hours	P-value (Baseline vs. 48 hours)	
Body weight (kg)	63.6 ± 17.5	61.36 ± 17.4	< 0.001	62.8 ± 15.4	60.2 ± 15.1	< 0.001	< 0.001

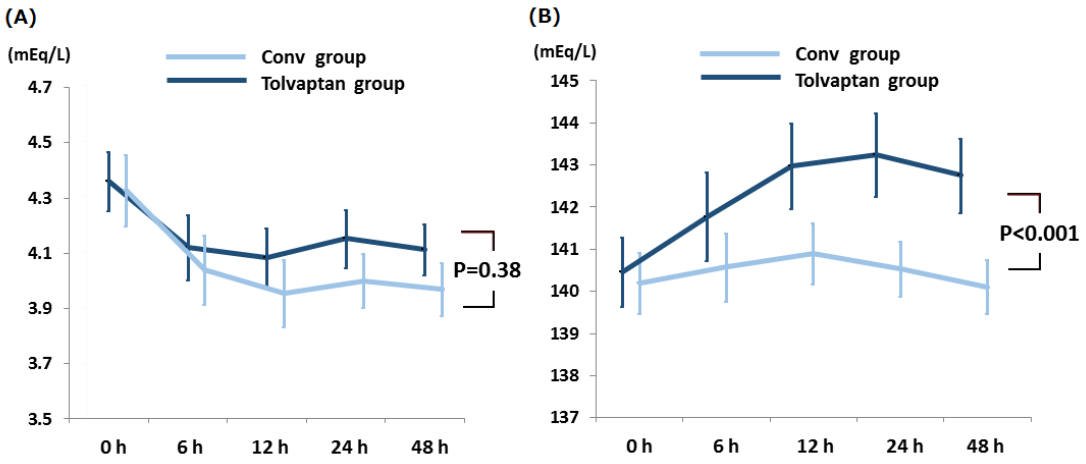
BNP (pg/dL)	729.8 (461.9 – 1482.2)	361.1 (173.9 – 694.3)	< 0.001	939.3 (532.9 – 1510.8)	468.5 (260.2 – 772.5)	< 0.001	0.60

Supplemental Table 3. The patients reported changes in dyspnea from baseline measured on a 7-point Likert scale

Dyspnea with 7-point Likert scale	6 hours		12 hours		24 hours		48 hours	
	Conv	Tol	Conv	Tol	Conv	Tol	Conv	Tol
Markedly improved	4 (3.7)	4 (3.7)	1 (0.9)	4 (3.7)	3 (2.8)	6 (5.6)	8 (7.5)	15 (13.9)
Moderately improved	10 (9.3)	15 (13.9)	19 (17.9)	36 (33.6)	28 (26.2)	49 (45.4)	48 (45.3)	59 (54.6)
Mildly improved	51 (47.2)	58 (53.7)	58 (54.7)	52 (48.6)	59 (55.1)	38 (35.2)	31 (29.2)	26 (24.1)
No change	42 (38.9)	30 (27.8)	26 (24.5)	14 (13.1)	17 (15.9)	15 (13.9)	18 (17.0)	7 (6.5)
Mildly worsened	1 (0.9)	1 (0.9)	1 (0.9)	0	0	0	1 (0.9)	1 (0.9)
Moderately worsened	0	0	0	1 (0.9)	0	0	0	0
Markedly worsened	0	0	1 (0.9)	0	0	0	0	0

Conv, conventional group; Tol, tolvaptan group

Supplemental Figure 1. Serum potassium (Panel A) and serum sodium (Panel B) changes from baseline to 48 hours


(A)
(mEq/L)
Conv group
Tolvaptan group
4.7
4.5
4.3
4.1
3.9
3.7
3.5
0 h
6 h
12 h
24 h
48 h
P=0.38
(B)
(mEq/L)
Conv group
Tolvaptan group
145
144
143
142
141
140
139
138
137
0 h
6 h
12 h
24 h
48 h
P<0.001

Chapter 6

Early Treatment with Tolvaptan improves diuretic response in acute heart failure with renal dysfunction

Yuya Matsue
Jozine M. ter Maaten
Makoto Suzuki
Sho Torii
Satoshi Yamaguchi
Seiji Fukamizu
Yuichi Ono
Hiroyuki Fujii
Takeshi Kitai
Toshihiko Nishioka
Kaoru Sugi
Yuko Onishi
Makoto Noda
Nobuyuki Kagiyama
Yasuhiro Satoh
Kazuki Yoshida
Peter van der Meer
Kevin Damman
Adriaan A. Voors
Steven R. Goldsmith

Clin Res Cardiol 2017. doi: 10.1007/s00392-017-1122-1.

Abstract

Background

Poor response to diuretics is associated with worse prognosis in patients with acute heart failure (AHF). We hypothesized that treatment with tolvaptan improves diuretic response in patients with AHF.

Methods

We performed a secondary analysis of the AQUAMARINE open-label randomized study in which a total of 217 AHF patients with renal impairment (eGFR < 60 mL/min/1.73 m2) were randomized to either tolvaptan or conventional treatment. We evaluated diuretic response to 40 mg furosemide or its equivalent based on two different parameters: change in body weight and net fluid loss within 48 hours.

Results

The mean time from patient presentation to randomization was 2.9 hour. Patients with a better diuretic response showed greater relief of dyspnea and less worsening of renal function. Tolvaptan patients showed a significantly better diuretic response measured by diuretic response based both body weight [−1.16 (IQR, −3.00 to −0.57) kg/40 mg vs. −0.51 (IQR, −1.13 to −0.20) kg/40 mg; P < 0.001] and net fluid loss [2125.0 (IQR, 1370.0–3856.3) mL/40 mg vs. 1296.3 (IQR, 725.2–1726.5) mL/40 mg; P < 0.001]. Higher diastolic blood pressure and use of tolvaptan were independent predictors of a better diuretic response.

Conclusions

Better diuretic response was associated with greater dyspnea relief and less WRF. Early treatment with tolvaptan significantly improved diuretic response in AHF patients with renal dysfunction.

Introduction

Volume overload and subsequent congestion are the primary causes and treatment targets for acute heart failure (AHF)[1,2]. Diuretics have therefore been the mainstay of treatment of patients with AHF[3]. Recent studies however have suggested that there are patients with AHF who may be refractory to conventional diuretic therapy[4,5]. This poor diuretic response is a strong and independent predictor of unfavorable prognosis[6], and no therapy has yet been proven to benefit patients with a poor diuretic response.

Tolvaptan is an oral, non-peptide, selective vasopressin-2 receptor antagonist, and prevents the activation of the aquaporin system and impairs the ability of the kidney to reabsorb water; as a result, free water excretion is increased. In the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan (EVEREST) trial, tolvaptan showed a favorable short-term effect but neutral long-term effect in AHF patients[7]. However, in this trial AHF patients were enrolled relatively late after presentation as a consequence of inclusion criteria (< 48 hours from hospitalization). Recent AHF studies have showed “time to treatment” is a factor associated with drug efficacy and patient prognosis[8,9] and the latest European Society of Cardiology heart failure guideline emphasizes the importance of treating AHF patients as quickly as possible[10]. Therefore, treatment with tolvaptan in the very early phase worth evaluating. Moreover, no study has evaluated diuretic response in Asian AHF patients. In the AQUAMARINE study (a randomized study evaluated efficacy of tolvaptan in patients with AHF and renal dysfunction), all patients were randomized within 6 hours from hospitalization. Consequently, median time from first presentation to randomization was 2.1 hour. In this study, we aimed to evaluate the effect of early treatment with tolvaptan on diuretic response in AHF patients with concomitant renal dysfunction.

Methods

Study population

This is a retrospective secondary analysis of the AQUAMARINE study. The study design and primary results of AQUAMARINE have been described elsewhere[11,12]. In brief, 217 patients with AHF and renal dysfunction (estimated glomerular filtration rate, 15–60 mL/min/1.73m^2) were randomized within 6 hours from hospitalization into two groups, either tolvaptan treatment or conventional treatment, to evaluate the efficacy of early treatment with tolvaptan. Fifty-three patients (48.6%) in the tolvaptan group received tolvaptan for more than 2 days, and no patient who was initially allocated to conventional group crossed over to tolvaptan during the first 48 hours. The protocol of the study was approved by the ethics committees of all participating centers, and written informed consent was obtained from all the participants. This trial was registered at UMIN-CTR (Unique identifier: UMIN000007109).

Data collection

In the AQUAMARINE study, data regarding blood pressure, heart rate, and improvement in dyspnea from baseline and blood samples were collected at 6, 12, 24, and 48 hours from enrollment. Dyspnea was assessed according to

the patient-reported seven-point Likert scale. Within 48 h, the amount of furosemide-equivalent loop diuretics, change in body weight from baseline, and urine output were noted down. Worsening renal function was defined as an increase of ≥0.3 mg/dL in the serum creatinine from the baseline at various pre-specified time points (6, 12, 24, and 48 hours from randomization). The incidence of the combined endpoints for all-cause mortality and re-hospitalization for heart failure within 90 days was also evaluated.

Diuretic response

We defined diuretic response as the change in body weight (kg) from baseline to 48 hours per 40 mg intravenous furosemide administration. Oral furosemide was converted to half the dose of intravenous furosemide. The doses of oral loop diuretics that were considered equivalent to 40 mg intravenous furosemide were 10 mg torasemide and 60 mg azosemide[13,14]. We also performed analyses by using net fluid loss within 48 hours as a measure of diuretic response. Diuretic response was measured according to body weight change in 189 cases after excluding 28 cases due to missing data on the total diuretic dose (n = 3) and body weight change (n = 25). Data on diuretic response based on net fluid loss were achieved in 171 cases and missing in 46 cases due to unavailability of information on water intake in 45 cases and on furosemide dose in 3 cases.

Statistical analysis

Data were expressed as mean ± standard deviation for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed data. Categorical data were expressed as numbers and percentages. The relationships between baseline characteristics, outcomes and tertiles of diuretic response were compared by one-way analysis of variance, Kruskal–Wallis test, or χ^2 test, as appropriate. Correlation analysis was performed using Spearman's rho. When necessary, variables were transformed for further analyses. Stepwise multiple linear regression analysis was performed using backward elimination method after including all variables with P values below 0.10 in the univariate analysis. Statistical analyses were performed using R version 3.1.2.

Results

In the AQUAMARINE study, 220 patients were originally enrolled, of which 217 were analyzed because one patient in the tolvaptan group and one patient in the conventional group withdrew their consent and data were missing for one patient in the tolvaptan group. The baseline characteristics of randomized patients were shown elsewhere[12]. The median age of the patients was 75 years (interquartile range [IQR], 68 – 81 years), and 64.9% was male. The median left ventricular ejection fraction was 44.5%, and 82 (37.8%) patients had a left ventricular ejection fraction ≥ 50%. Mean baseline eGFR was 40.5 mL/min/1.73 m2, and 57 (26.3%) patients had an eGFR < 30 mL/min/1.73 m2. Time from first-medical record input to randomization was obtained in 210 (96.8%) patients, and it was 2.9 hours in mean, and 2.1 hours in median. Time from patient appearance to randomization and the place they appear was shown in Supplemental Figure 1.

During the first 48 hours from study enrollment, the median administered amount of furosemide-equivalent diuretic dose was 100 mg (IQR, 62.5–160 mg), median total body weight change was −2.30 kg (IQR, −3.50 to −1.18 kg), and median net fluid loss was 3973.0 mL (IQR, 2566.3–5410.0 mL). The median values for the measures of diuretic response were −0.83 (IQR, −1.50 to −0.40) kg/40mg body weight and 1582.8 (IQR, 895–2478.3) mL/40mg net fluid loss. The baseline characteristics of the study population according to diuretic response tertiles are shown in Table 1. Using baseline characteristics, poor diuretic response based on change in body weight, was associated with less edematous status, less history of hypertension, and more hyponatremia. These associations were retained for diuretic response based on net fluid loss. In correlation analysis, change in body weight and net fluid loss showed a statistically significant, but relatively weak correlation (Spearman's rho = −0.47, $P < 0.001$) (Supplemental Figure 2).

Table 1. Baseline characteristics and relationship among tertiles of diuretic response

Diuretic response (per 40mg furosemide-equivalent) [min-max]	Diuretic response with body weight changes (kg/40mg furosemide)				Diuretic response with Net-fluid loss (mL/40mg furosemide)			
	Tertile1 (Best) (N=66)	Tertile2 (N=61)	Tertile3 (Worst) (N=62)	P value	Tertile1 (Best) (N=57)	Tertile2 (N=57)	Tertile3 (Worst) (N=57)	P value
	-2.42 [-10.6 – -1.20]	-0.80 [-1.20 – -0.50]	-0.21 [-0.48 – 4.00]		4427.5 [2875.0 – 21520.0]	2046.9 [1634.0 –2843.3]	1009.3 [98.2 – 1577.1]	
Age	72±8	72±12	74±9	0.456	73±8	73±9	71±11	0.498
Male (%)	46 (69.7)	42 (68.9)	37 (59.7)	0.421	37 (64.9)	41 (71.9)	35 (61.4)	0.482
Body weight at baseline	63.0 (56.0-69.7)	60.0 (51.0-68.7)	60.1 (49.9-69.5)	0.409	63.0 (55.9-68.3)	61.8 (55.0-71.4)	64.1 (52.6-70.3)	0.975
SBP (mmHg)	141±26	139±31	137±25	0.767	144±24	140±29	135±26	0.158
DBP (mmHg)	83±19	78±22	77±19	0.188	84±18	78±18	78±18	0.113
HR (bpm)	96±30	89±25	90±24	0.213	94±28	92±24	91±25	0.808
Edema at baseline* (%)				0.074				0.376
None	6 (9.1)	8 (13.1)	11 (18.0)		5 (8.8)	8 (14.0)	7 (12.5)	
Mild	15 (22.7)	25 (41.0)	23 (37.7)		15 (26.3)	13 (22.8)	21 (37.5)	
Moderate	23 (34.8)	16 (26.2)	18 (29.5)		19 (33.3)	24 (42.1)	18 (32.1)	
Severe	22 (33.3)	12 (19.7)	9 (14.8)		18 (31.6)	12 (21.1)	10 (17.9)	
Edema moderate/severe at baseline (%)	45 (68.2)	28 (45.9)	27 (44.3)	0.01	37 (64.9)	36 (63.2)	28 (50)	0.212
Orthopnea at baseline (%)	48 (72.7)	35 (57.4)	37 (59.7)	0.149	39 (68.4)	39 (68.4)	38 (66.7)	0.974

Pulmonary congestion at baseline (%)	64 (97.0)	53 (86.9)	60 (96.8)	0.031	55 (96.5)	50 (87.7)	55 (96.5)	0.088
NYHA III/IV (%)	48 (72.7)	36 (59.0)	39 (62.9)	0.245	42 (73.7)	39 (68.4)	42 (73.7)	0.771
Ischemic etiology (%)	15 (22.7)	13 (21.3)	20 (32.3)	0.313	16 (28.1)	13 (22.8)	15 (26.3)	0.807
LVEF (%)	43.3±17.5	48.7±16.1	47.7±17.3	0.161	46.2±19.1	43.7±17.1	47.8±15.9	0.443
Medical history (%)								
HF admission	26 (39.4)	26 (42.6)	32 (51.6)	0.358	28 (49.1)	21 (36.8)	28 (49.1)	0.314
Hypertension	56 (84.8)	41 (67.2)	50 (80.6)	0.046	52 (91.2)	35 (62.5)	43 (75.4)	0.001
Diabetes	29 (43.9)	23 (37.7)	30 (48.4)	0.486	27 (47.4)	24 (42.1)	24 (42.1)	0.808
Dyslipidemia	33 (50.0)	19 (31.1)	29 (46.8)	0.116	28 (49.1)	24 (42.1)	19 (33.3)	0.288
Atrial fibrillation	38 (57.6)	31 (50.8)	32 (51.6)	0.596	30 (52.6)	31 (54.4)	32 (56.1)	0.705
Smoking (Current or Ex)	43 (65.2)	38 (63.3)	33 (55.0)	0.467	34 (60.7)	38 (67.9)	31 (58.5)	0.57
Drugs at admission (%)								
ACE-I	6 (9.1)	7 (11.5)	1 (1.6)	0.092	6 (10.5)	4 (7.0)	4 (7.0)	0.733
ARB	19 (28.8)	16 (26.2)	27 (43.5)	0.085	18 (31.6)	13 (22.8)	20 (35.1)	0.336
Beta blocker	25 (37.9)	21 (34.4)	25 (40.3)	0.795	21 (36.8)	19 (33.3)	27 (47.4)	0.279
Aldosterone antagonist	14 (21.2)	9 (14.8)	18 (29.0)	0.157	11 (19.3)	11 (19.3)	8 (14.0)	0.695
Digoxin	4 (6.1)	3 (4.9)	4 (6.5)	0.931	4 (7.0)	3 (5.3)	3 (5.3)	0.899
Diuretics	27 (40.9)	23 (37.7)	28 (45.2)	0.701	20 (35.1)	25 (43.9)	24 (42.1)	0.6
Furosemide equivalent dose among users (mg)	40 (5-80)	40 (10-200)	40 (10-120)	0.356	40 (10-80)	20 (10-120)	40 (5-200)	0.575
Tolvaptan treatment (%)	47 (71.2)	33 (54.1)	17 (27.4)	<0.001	46 (80.7)	26 (45.6)	19 (33.3)	<0.001

IV therapy within 48 hours (%)								
Carperitide	25 (37.9)	23 (37.7)	19 (30.6)	0.628	25 (43.9)	24 (42.1)	12 (21.1)	0.018
Dopamine	1 (1.5)	0 (0.0)	2 (3.2)	0.359	0 (0.0)	2 (3.5)	0 (0.0)	0.132
Dobutamine	5 (7.6)	5 (8.2)	9 (14.5)	0.359	3 (5.3)	7 (12.3)	8 (14.0)	0.271
Nitrate	16 (24.2)	10 (16.4)	9 (14.5)	0.321	16 (28.1)	8 (14.0)	11 (19.3)	0.172
Vasodilator	17 (25.8)	10 (16.4)	11 (17.7)	0.359	16 (28.1)	9 (15.8)	13 (22.8)	0.286
Heparin	34 (51.5)	19 (31.1)	25 (40.3)	0.065	32 (56.1)	17 (29.8)	24 (42.1)	0.018
Lab data at baseline								
Creatinine	1.5±0.6	1.3±0.5	1.5±0.5	0.148	1.4±0.5	1.4±0.5	1.5±0.7	0.728
eGFR	38.3±13.9	43.5±12.2	38.9±13.4	0.056	39.1±13.6	42.0±13.2	40.2±14.2	0.503
BUN	28 (20-35)	26 (19-35)	28 (20-37)	0.891	24 (19-32)	28 (18-35)	28 (22-35)	0.466
Sodium	141±4	141±3	139±4	0.017	141±4	140±4	139±5	0.149
Potassium	4.4±0.6	4.2±0.5	4.4±0.7	0.288	4.4±0.6	4.3±0.5	4.3±0.8	0.583
BNP	939.3 (544.4-1477.6)	866.9 (492.0-1554.1)	750.2 (393.5-1463.6)	0.373	897.9 (572.1-1622.0)	726.3 (491.0-1094.6)	1009.1 (393.5-1716.2)	0.189
Dose of diuretics use within 48 hours (mg)	60 (23-100)	100 (80-140)	140 (100-200)	<0.001	60 (20-80)	100 (80-140)	160 (110-220)	<0.001
Urine volume within 48 hours (mL)	6584.1±3559.0	6192.2±2399.2	4500.3±1679.1	<0.001	7319.9±3599.0	5974.9±1877.2	4399.8±1906.5	<0.001
Water intake within 48 hours (mL)	1867.3±1434.1	1685.3±986.4	1446.0±933.4	0.183	1902.5±1456.2	1497.4±739.4	1488.3±1054.2	0.082
Net fluid loss within 48 hours (mL)	4747.9±2839.2	4419.8±1999.0	3344.2±1594.1	0.006	5417.3±2768.2	4477.5±1718.9	2911.5±1675.1	<0.001

Body weight changes within 48 hours (kg)	-3.8 (-5.3--2.6)	-2.4 (-2.9--1.6)	-0.9 (-1.5-0.0)	<0.001	-3.0 (-4.7--2.0)	-2.4 (-3.3--1.3)	-1.4 (-2.5--0.8)	<0.001

* Data on edema at baseline was missing in one patient.

To identify predictors of diuretic response, univariable (Supplemental Table 1) and multivariable linear regression analysis (Table 2) for both parameters were performed. The only independent predictors of a good diuretic response for both criteria were tolvaptan use and a higher diastolic blood pressure. There was no interaction between baseline diuretics and tolvaptan on diuretic response for both BW definition (P value for interaction = 0.816) and net fluid loss definition (P value for interaction = 0.642). Likewise, no significant interaction was observed between baseline sodium level, renal function, and impact of tolvaptan treatment on diuretic response (all P value for interaction > 0.20). For both diuretic response definitions, no interaction was found on the effect of tolvaptan on diuretic response between patients who were treated with and without carperitide (P for interaction = 0.137 with body weight definition and 0.707 with net fluid loss definition).

Table 2. Multivariable linear regression analysis of diuretic response

Variable	Standardized Beta	t	P value
Diuretic response with body weight changes (kg/40mg furosemide) Adjusted R2=0.214			
Tolvaptan treatment	-0.339	-5.246	<0.001
Heparin IV	-0.241	-3.707	<0.001
DBP	-0.149	-2.279	0.024
Edema (moderate/severe)	-0.137	-2.088	0.011
Diuretic response with Net fluid loss (mL/40mg furosemide) Adjusted R2=0.176			
Tolvaptan treatment	0.387	5.495	<0.001
DBP	0.199	2.854	0.005

Patients with a poor diuretic response were less likely to have an improvement in dyspnea relief within 48 hours from randomization, as defined by moderate or marked improvement from baseline according to the seven-point Likert scale (Table 3). A poor diuretic response was also significantly associated with more WRF (Table 3). A worse diuretic response was not associated with an increased risk of pre-specified prognostic endpoints within 90 days.

Table 3. Outcomes of the tertiles of diuretic response

Diuretic response (per 40mg furosemide-equivalent) [min-max]	Diuretic response with body weight changes (kg/40mg furosemide)				Diuretic response with Net-fluid loss (mL/40mg furosemide)			
	Tertile1 (Good) (N=66) -2.42 [-10.6--1.20]	Tertile2 (N=61) -0.8 [-1.20--0.50]	Tertile3 (N=62) -0.21 [-0.48-4.00]	P value	Tertile1 (Good) (N=57) 4427.5 [2875.0-21520.0]	Tertile2 (N=57) 2046.9 [1634.0-2843.3]	Tertile3 (N=57) 1009.3 [98.2-1577.1]	P value
Dyspnea relief (moderately or markedly)								
6 hours	15 (22.7)	10 (16.4)	8 (12.9)	0.331	14 (24.6)	8 (14.0)	8 (14.0)	0.223
12 hours	25 (39.1)	20 (32.8)	10 (16.4)	0.017	24 (42.9)	18 (31.6)	11 (19.3)	0.026
24 hours	35 (53.0)	31 (51.7)	12 (19.4)	<0.001	33 (57.9)	29 (50.9)	12 (21.1)	<0.001
48 hours	52 (80.0)	44 (72.1)	21 (34.4)	<0.001	47 (83.9)	44 (78.6)	18 (31.6)	<0.001
WRF (Cre increase ≥0.3mg/dL from baseline) (%)	11 (16.7)	13 (21.3)	22 (35.5)	0.037	8 (14.0)	13 (22.8)	22 (38.6)	0.009
Length of hospital stay (Days)	13.9 (8.4-18.7)	13.2 (8.5-19.4)	18.4 (10.3-27.2)	0.101	13.5 (8.9-17.4)	13.4 (10.0-20.6)	18.4 (11.2-24.4)	0.088
Prognosis within 90 days (%)								
Death	1 (1.5)	2 (3.3)	4 (6.5)	0.335	1 (1.8)	3 (5.3)	3 (5.3)	0.551
Combined of death or HF readmission	7 (10.8)	9 (14.8)	9 (14.5)	0.759	4 (7.0)	9 (15.8)	9 (15.8)	0.271

Figure 1 shows the diuretic response according to randomization group, i.e. with and without tolvaptan treatment. Compared to patients who were not treated with tolvaptan, those who were treated with tolvaptan showed a significantly better diuretic response based on assessment by both body weight change [−1.16 (IQR, −3.00 to −0.57) kg/40 mg vs. −0.51 (IQR, −1.13 to −0.20) kg/40 mg; P < 0.001] and net fluid loss [2125.0 (IQR, 1370.0 to 3856.3) mL/40 mg vs. 1296.3 (IQR, 725.2 to 1726.5) mL/40 mg; P < 0.001].

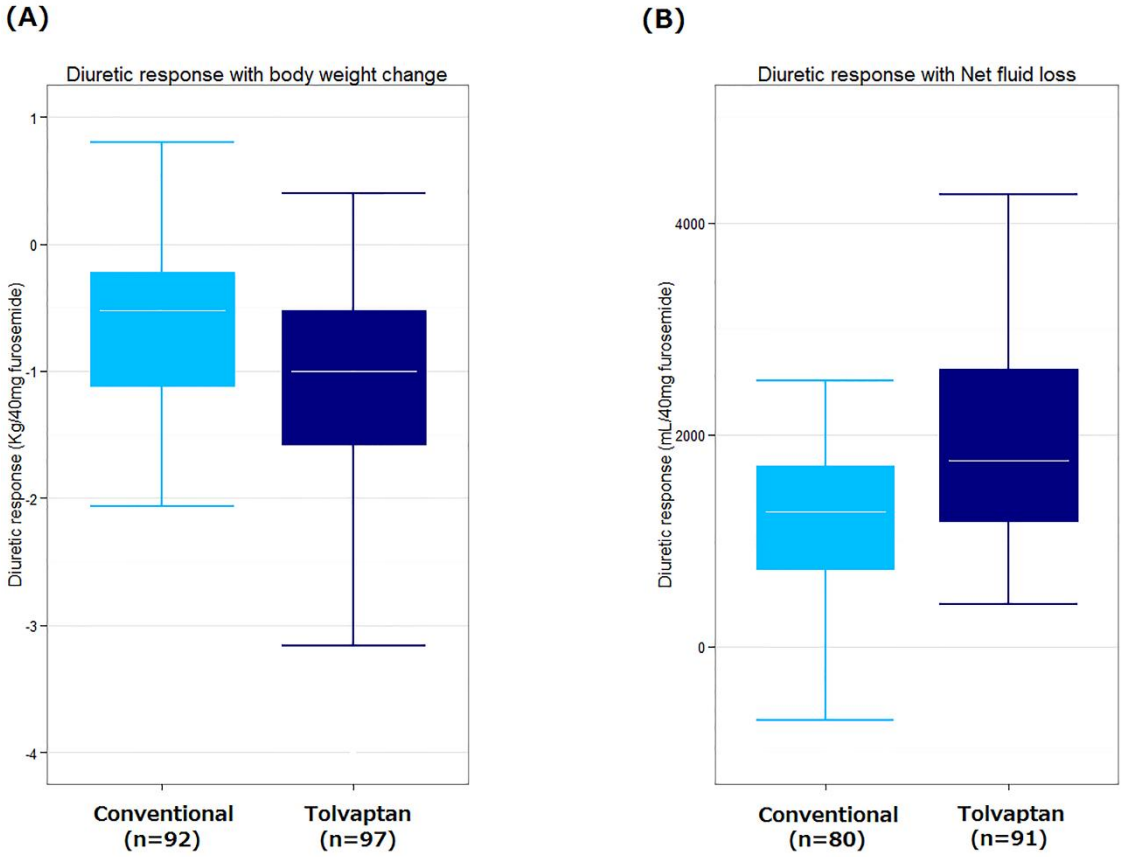


Figure 1. Diuretic response in patients with acute heart failure according to treatment with tolvaptan Measurements compared were (A) change in body weight and (B) net fluid loss

Discussion

In patients with AHF and renal dysfunction, very early treatment with tolvaptan was independently associated with better diuretic response. AHF patients with poor diuretic response had less dyspnea relief and more frequently experienced worsening renal function.

Diuretic response in AHF

In spite of the lack of a universal definition, poor response to diuretic therapy has been shown to be one of the most powerful prognostic predictors in patients with heart failure[4,13,15,16]. Initial studies used diuretic dose to define diuretic response, i.e., patients with persistent heart failure despite treatment with a certain dose of diuretics were defined to have a “poor diuretic response”[15,17]. However, this definition used only amount of

diuretics and hereby obviously ignored response to the diuretics and therefore assumed equal effectiveness. Recently, a novel definition of diuretic response based on urine/body weight response to a certain amount of diuretics was proposed[6]. In all studies that evaluated its prognostic potential, diuretic response consistently showed significant prognostic ability in patients with AHF when this modified definition was used[4,5,18,19].
There has been no consensus on the parameter used to measure diuretic response to 40 mg furosemide or its equivalent, although recent studies have used either change in body weight, net fluid loss, or total urinary output. In the present study, we showed relatively poor correlation between the two measures of diuretic response. This result was in line with that of the DOSE trial and ASCEND-HF, which demonstrated a poor agreement between net fluid loss and weight loss[5,20]. It is clear that we need better measures of diuretic response to encompass natriuretic response, change in volume distribution, and change in hemodynamic status. However, our consistent results on the improvement of diuretic response, by two different parameters, with tolvaptan supported our hypothesis.

According to this novel definition, the median diuretic response was −0.51 kg/40 mg/48 hours furosemide in the conventional group in our study. This was greater than approximately 0.4 kg/40 mg of furosemide-equivalent diuretic response in the patients of the ASCEND-HF (weight change from admission to 48 hours), RELAX-AHF (weight change from day1 to 5), and PROTECT (weight change from day1 to 4) studies[4,5,18]. This better diuretic response in this AQUAMARINE cohort did not match our expectations because our study included only AHF patients with renal dysfunction on admission and earlier studies suggest that renal dysfunction predisposes to worse diuretic response[4,5,18]. There are several conceivable speculations for this unexpected result. First, lower doses of loop diuretic were given in AQUAMARINE, compared to other studies and the additional effect of a drug usually decreases at higher doses. Second, although baseline creatinine values were higher in the AQUAMARINE cohort than in the ASCEND-HF cohort, levels of baseline blood urea nitrogen were not substantially different between these two studies. Given that blood urea nitrogen, but not creatinine, has been suggested by previous studies as the most powerful determinant of diuretic response[4,21], this may be one of the reasons for discrepancy in our study. Third, median time till randomization from patient arrival was 2.1 hours and 41.4% of all AQUAMARINE cohort was randomized before admission at the emergency department or clinic. This is surprisingly short given that mean time from admission to randomization was 15.5 hours in ASCEND-HF and 7.9 hours in RELAX-AHF[22]. This means AQUAMARINE randomized AHF patients much earlier, and we could therefore evaluate diuretic response in the very early phase which was not possible with previous diuretic response studies in AHF cohorts. This difference in the time window might be associated with the unexpected good diuretic response in our study cohort. Finally, our results lead to hypothesis that there may be a racial difference in diuretic response. All of the studies regarding diuretic response so far predominantly enrolled Western AHF patients and little is currently known about diuretic response in Asian AHF patients. This hypothesis is supported by the observation that the amount of intravenous loop diuretics used in the acute phase was very low (around or less than 100mg/48 hours) in Japanese AHF patients compared to Western patients[12,23]. Therefore, influence of racial and/or genetic information on diuretic response needs to be elucidated in future studies.

For both diuretic response parameters, high blood pressure was associated with a good diuretic response. These findings were in accordance with the results of previous studies. In the PROTECT, RELAX-AHF, and ASCEND-HF cohorts, low diastolic blood pressure was an independent predictor of poor diuretic response[4,5,18]. Interestingly, intravenous unfractionated heparin was associated with good diuretic response measured with body weight. We have no clear explanation for this finding; however, hyperkalemia is known to be a rare but possible complication of heparin therapy[24], and hypokalemia was suggested as an independent predictor of poor diuretic response in PROTECT. Moreover, there is a case report that suggests a direct effect of heparin on diuresis in patients with AHF[25]. The association between intravenous heparin and diuretic response needs to be precisely elucidated in the future studies.

Poor diuretic response was significantly associated with a high incidence of worsening renal function and low rate of improvement in dyspnea at almost all time points. These results are in line with the findings of previous studies[4,18]; however, it should be acknowledged that the number of events were very small and this study was obviously underpowered to evaluate prognostic significance of diuretic response.

Effect of tolvaptan on diuretic response

Although some interventions to treat AHF patients with diuretic resistance have been investigated, there has been no proven therapy to improve diuretic resistance in this high risk population. In the ROSE-AHF study, neither low-dose nesiritide nor low-dose dopamine on top of standard of therapy was associated with a greater reduction in body weight within 72 hours[26]. Given that the total amount of furosemide-equivalent diuretic used within 72 hours was not significantly different, neither low-dose dopamine nor low-dose nesiritide was suggested to improve diuretic response. Likewise, in ASCEND-HF, nesiritide did not improve diuretic response[5]. In RELAX-AHF, serelaxin did not show a significant improvement in diuretic response of patients with AHF despite its potentially favorable effects on prognosis [18,27,28]. Rolofylline, an adenosine A1-receptor antagonist, on the other hand did improve diuretic response[4]. However, its clinical use was hampered by a neutral effect on prognosis and the concern for neurological adverse events. Ultrafiltration might be a promising decongestive strategy[29]; however, it has not been studied specifically in patients with a poor diuretic response.

In the present study, we showed that very early treatment with tolvaptan could improve diuretic response in AHF patients with renal impairment. The pathophysiological background of this favorable effect of tolvaptan on diuretic response remains to be elucidated; however, it may be attributed to certain differences in the mechanisms of action between loop diuretics and tolvaptan. First, time-dependent diuretic resistance was observed with loop diuretics. In patients who have been treated with diuretics for a long time, effectiveness is blunted gradually with time[30]. Second, loop diuretics have to be bound to plasma albumin and delivered to the proximal tubules in order to exert their effects. Therefore, hypoalbuminemia, which is common in patients with AHF, could contribute to poor diuretic response[31,32]. Third, active secretion of loop diuretics into the lumen via an organic acid transporter is needed

for them to act[33]. This transporter could be inhibited by endogenous organic anions[34]. However, compared with furosemide, tolvaptan has a different mechanism of action, i.e., inhibiting the activation of vasopressin-2 receptor by arginine–vasopressin and subsequent insertion of aquaporin-2 channels in the collecting tubules. This might be one of the reasons for the improvement in diuretic response in renal-impaired patients with AHF after intake of tolvaptan.

Contrary to our result, recent sub-analysis from EVEREST showed a lack of significant difference in prescription rate of tolvaptan between good/bad diuretic response groups[35]. There are some differences in patient backgrounds between EVEREST and AQUAMARINE that possibly explain this discordance (e.g. racial difference, baseline renal function). However, the most conceivable explanation for this discrepancy is time to treatment. In EVEREST, time from hospitalization to dyspnea assessment (the next calendar day after the first drug administration) was more than 36 hours in 47.7%, and more than 60 hours in 20.2%[36]. In AQUAMARINE about 40% of all patients were randomized before admission to the hospital ward and this early capture of AHF patients may lead to short time to randomization and better diuretic response. The association between time to therapy and diuretic response in AHF patient needs to be addressed in future studies.

Tolvaptan is expected to cause aquaresis but not natriuresis. As sodium retention plays a pivotal role in pathophysiology of AHF, aquaresis may have a different impact on prognosis from natriuresis in AHF patients. Although the pathophysiological background of the association between diuretic response and prognosis has yet to be elucidated, early successful decongestion and subsequent symptom relief are plausible mechanisms. Given that several studies, including AQUAMARINE, have consistently showed urine output with tolvaptan (i.e. aquaresis) could also lead to decongestion and subsequent symptom relief, improvement of diuretic response with early treatment with tolvaptan in AHF patients potentially improves outcome. From this perspective, EVEREST might not be suitable to evaluate this hypothesis as tolvaptan was used relatively late and did not improve diuretic response. As we showed improvement in diuretic response with very early treatment with tolvaptan for the first time, future studies on early use of tolvaptan for patients with AHF having poor diuretic response are warranted.

Our study had several limitations; primarily, its open-label design, which could have influenced some subjective prognostic variables, including relief of dyspnea. This study focused on short-term responses and did not have sufficient power to detect long term differences in WRF. We could not address the association between diuretic response and prognosis because of very little number of events. As we recruited and randomized patients very early in our study, some non-AHF patients might have been included. However, all patients went through careful clinical history taking, physical examination, chest X-ray and analysis of natriuretic. Only after confirmation that patients met the criteria as stated in the protocol, they were randomized and received the study drug. In addition, we performed sensitivity analyses comparing the effects of tolvaptan in patients with a BNP between 100 – 350 pg/ml and above 350 pg/mL We found no interaction in the effect of tolvaptan on diuretic response in patients with higher versus lower BNP levels at admission (P value for interaction = 0.183). No standardized diuretic regimen was applied and usage of diuretics was at the discretion of the treating physician. Our findings regarding association

between diuretic response and dyspnea relief should be interpreted carefully because baseline severity of dyspnea was not evaluated and difference in baseline dyspnea severity between good and poor diuretic response group might affect difference in degree of dyspnea relief.

The most powerful limitation of this study which should be acknowledged is that this is a post-hoc and non-pre-specified analysis. Moreover, several analyses were performed without adjusting for multiple testing. Given these points, our study result should be interpreted as an exploratory analysis and hypothesis generating.

Conclusions

Very early treatment with tolvaptan improved diuretic response in patients with a hospital admission for AHF. Future research focusing on the prognostic implication of improving diuretic response with early treatment with tolvaptan in patients with poor diuretic response is warranted.

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Supplemental materials

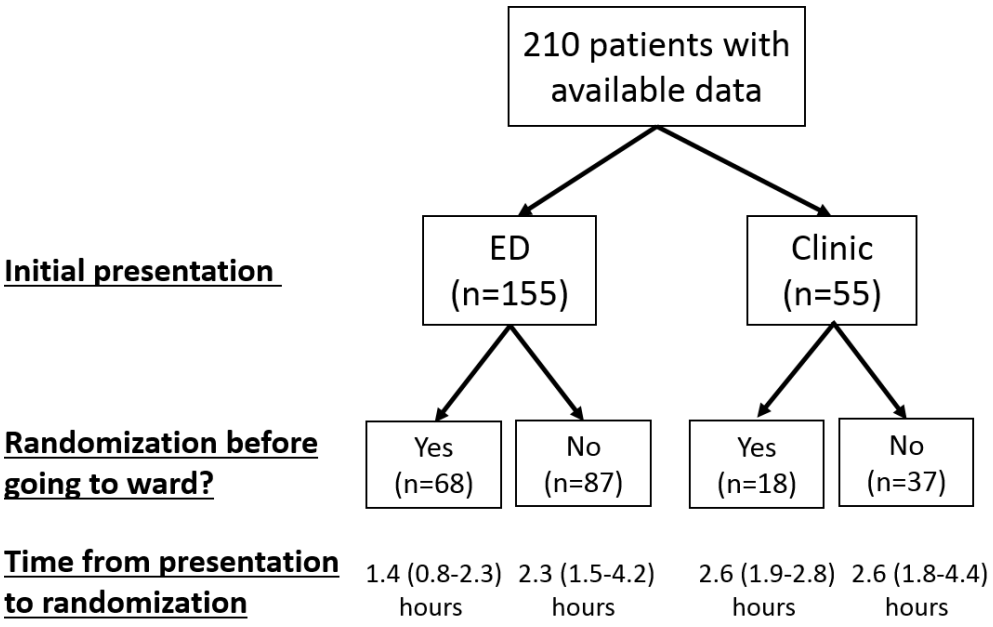
Supplemental Table 1. Univariable linear regression analysis for diuretic response

Variables	Diuretic response with body weight changes (kg/40mg furosemide)			Diuretic response with Net fluid loss (mL/40mg furosemide)		
	Standardized beta	t	P value	Standardized beta	t	P value
Age (years)	0.04	0.548	0.585	-0.009	-0.121	0.904
Male (%)	-0.085	-1.169	0.244	0.003	0.045	0.964
SBP (mmHg)	-0.157	-2.177	0.031	0.156	2.047	0.042
DBP (mmHg)	-0.196	-2.73	0.007	0.152	1.982	0.049
Heart rate (bpm)	-0.12	-1.654	0.099	0.051	0.656	0.513
Left ventricular ejection fraction (%)	0.125	1.710	0.089	-0.055	-0.71	0.478
NYHA III/IV (%)	-0.046	-0.629	0.531	-0.001	-0.011	0.991
Tolvaptan treatment	-0.349	-5.092	<0.001	0.404	5.711	<0.001
Medical History (%)						
HF admission	0.079	1.086	0.279	0.016	0.2	0.841
Hypertension	-0.097	-1.333	0.184	0.100	1.299	0.196
Diabetes	-0.032	-0.433	0.666	0.001	0.009	0.993
Dyslipidemia	0.009	0.126	0.9	0.024	0.309	0.757
Atrial fibrillation	-0.025	-0.335	0.738	-0.018	-0.229	0.819
Smoking (Current or Ex)	-0.135	-1.843	0.067	0.042	0.534	0.594
Drug at admission (%)						
Furosemide equivalent dose (mg)	0.065	0.891	0.374	-0.139	-1.813	0.072
ACE	-0.042	-0.573	0.567	0.024	0.298	0.766
ARB	0.083	1.141	0.255	-0.055	-0.714	0.476
Beta blocker	-0.002	-0.025	0.98	-0.07	-0.913	0.362
Aldosterone antagonist	0.073	0.996	0.321	0.044	0.566	0.572
Digoxin	0.025	0.338	0.735	-0.018	-0.231	0.817
Time to Randomization (hour)	-0.113	-1.532	0.127	0.015	0.192	0.848
IV therapy w/i 48h (%)						
Carperitide	-0.146	-2.02	0.045	0.195	2.574	0.011
Nitrate	-0.217	-3.041	0.003	0.137	1.787	0.076
ISDN	0.045	0.611	0.542	-0.091	-1.184	0.236

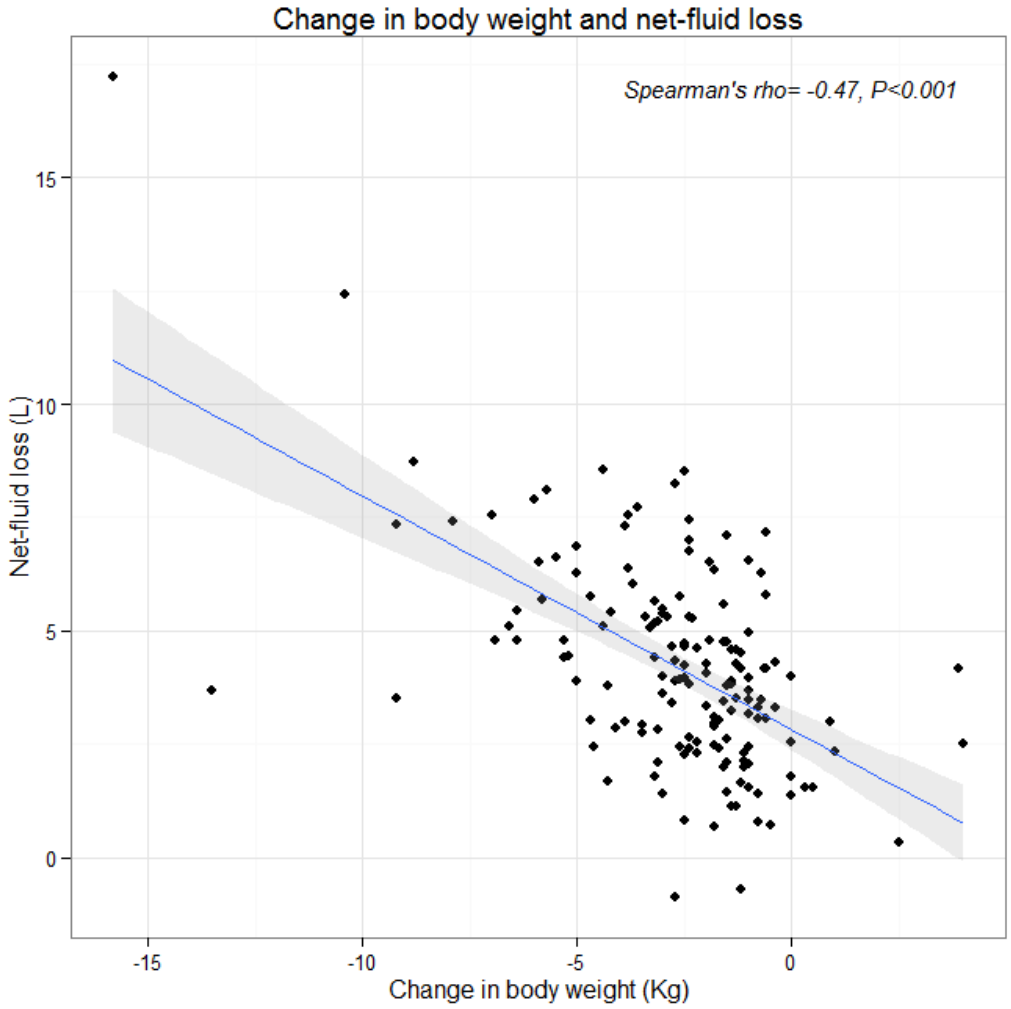
Vasodilator	-0.195	-2.715	0.007	0.103	1.335	0.184
Nicorandil	0.016	0.214	0.831	-0.089	-1.149	0.252
Heparin	-0.233	-3.275	0.001	0.109	1.428	0.155
Dopamin	0.026	0.357	0.722	0.003	0.045	0.964
Dobutamin	0.117	1.619	0.107	-0.129	-1.683	0.094
Lab data						
Creatinine (mg/dL)	-0.112	-1.542	0.125	-0.031	-0.408	0.684
eGFR (mL/min/1.73m2)	0.072	0.988	0.324	-0.032	-0.417	0.677
BUN (mg/dL)	-0.007	-0.101	0.919	-0.161	-2.107	0.037
Na (mEq/L)	-0.119	-1.643	0.102	0.164	2.154	0.033
K (mEq/L)	-0.066	-0.909	0.365	0.011	0.14	0.889
BNP (pg/mL)	-0.108	-1.479	0.141	-0.045	-0.584	0.56
Body weight at admission (Kg)	-0.037	-0.505	0.615	-0.002	-0.027	0.979
Edema (moderate/severe)	-0.191	-2.647	0.009	0.177	2.317	0.022
Orthopnea	-0.105	-1.448	0.149	0.046	0.601	0.549
Pulmonary Congestion	-0.048	-0.656	0.513	0.043	0.553	0.581
Water intake	-0.069	-0.854	0.395	0.169	2.21	0.029

* Time-to-randomization was missing in 3 patients

Supplemental Figure 1. Place of initial presentation, randomization timing, and time from presentation to randomization in the AQUAMARINE cohort



Supplemental Figure 2. Scatter plot between body weight change and net fluid loss with fit line and its 95% confidence interval (shaded area)



rho: Spearman's rank coefficient of correlation

Chapter 7

Time-to-Furosemide Treatment and Mortality in Patients Hospitalized with Acute Heart Failure

Yuya Matsue
Kevin Damman
Adriaan A. Voors
Nobuyuki Kagiyama
Tetsuo Yamaguchi
Shunsuke Kuroda
Takahiro Okumura
Keisuke Kida
Atsushi Mizuno
Shogo Oishi
Yasutaka Inuzuka
Eiichi Akiyama
Ryuichi Matsukawa
Kota Kato
Satoshi Suzuki
Takashi Naruke
Kenji Yoshioka
Tatsuya Miyoshi
Yuichi Baba
Masayoshi Yamamoto
Koji Murai
Kazuo Mizutani
Kazuki Yoshida
Takeshi Kitai

J Am Coll Cardiol 2017;69:3042-3051

Abstract

Background

Acute heart failure (AHF) is a life-threatening disease requiring urgent treatment, including a recommendation for immediate initiation of loop diuretics.

OBJECTIVES We prospectively evaluated the association between time-to-diuretic treatment and clinical outcome.

Methods

REALITY-AHF (Registry Focused on Very Early Presentation and Treatment in Emergency Department of Acute Heart Failure) was a prospective, multicenter, observational cohort study that primarily aimed to assess the association between time to loop diuretic treatment and clinical outcome in patients with AHF admitted through the emergency department (ED). Door-to-furosemide (D2F) time was defined as the time from patient arrival at the ED to the first intravenous furosemide injection. Patients with a D2F time <60 min were pre-defined as the early treatment group. Primary outcome was all-cause in-hospital mortality.

Results

Among 1,291 AHF patients treated with intravenous furosemide within 24 h of ED arrival, the median D2F time was 90 min (interquartile range: 36 to 186 min), and 481 patients (37.3%) were categorized as the early treatment group. These patients were more likely to arrive by ambulance and had more signs of congestion compared to the non-early treatment group. In-hospital mortality was significantly lower in the early treatment group (2.3% vs 6.0% in the non-early treatment group; p = 0.002). In multivariate analysis, earlier treatment remained significantly associated with lower in-hospital mortality (odds ratio: 0.39; 95% confidence interval: 0.20 to 0.76; p = 0.006).

Conclusions

In this prospective multicenter, observational cohort study of patients presenting at the ED for AHF, early treatment with intravenous loop diuretics was associated with lower in-hospital mortality.

Abbreviations and acronyms

AHF = acute heart failure

BNP = B-type natriuretic peptide

D2F = door-to-furosemide

ED = emergency department

GWTG-HF = Get With the Guidelines-Heart Failure

Acute heart failure (AHF) is a life-threatening disease that remains an important public health issue, with high morbidity, mortality, and economic burden. While a paradigm shift has occurred regarding treatment of chronic systolic heart failure (HF), the management of AHF has not changed for several decades, and most clinical studies investigating several drugs targeting this population have failed to demonstrate a favorable prognostic impact (1).
The emergency department (ED) is a major stage for hospitalized patients with AHF. In the United States, almost 1 million ED visits for AHF occur per year, with ≥80% of patients admitted (2,3). The importance of the ED phase in managing AHF has become increasingly apparent as recent post hoc studies have highlighted the fact that while patient characteristics are important, the efficacy of any intervention/treatment may be time dependent (4-6). Therefore, recent HF guidelines and recommendations emphasized the importance of immediate diagnosis and treatment of patients presenting with AHF (7,8). However, this concept has only been evaluated using retrospective data. REALITY-AHF (Registry Focused on Very Early Presentation and Treatment in Emergency Department of Acute Heart Failure), was a prospective multicenter study to evaluate the association between time to treatment and clinical outcome in AHF patients presenting at the ED.

METHODS

The main objective of REALITY-AHF was to determine the prognostic impact of time to treatments for AHF performed in the acute phase. All consecutive patients with AHF hospitalized through the ED at participating hospitals were enrolled upon the initial hospital admission. Only the first hospitalization during the study period was registered. Patients were included if they were age ≥20 years old and diagnosed with AHF in the ED within 3 h of their first evaluation by caregivers. The AHF diagnosis was made based on Framingham criteria (9). Exclusion criteria were: 1) treatment with an intravenous (IV) drug prior to ED arrival; 2) previous heart transplantation; 3) on either chronic peritoneal dialysis or hemodialysis; 4) acute myocarditis; and 5) acute coronary syndrome requiring emergent or urgent revascularization. Patients with missing B-type natriuretic peptide (BNP) or N-terminal-proBNP (NT-proBNP) data, and patients with a BNP level <100 pg/ml or NT-proBNP level <300 pg/ml at baseline were also excluded. Enrollment was performed from August 2014 to December 2015. Among the 20 participating hospitals, 9 were university hospitals and 11 were nonuniversity teaching hospitals.
Since all patients were enrolled at the ED, the time of ED arrival was recorded, and data were collected for up to 48 h from the time of ED arrival. Drug and doses of all IV treatments were recorded. Additionally, the time from arrival to the “first IV furosemide” administration was recorded for all patients as it was the primary pre-specified variable of interest. Oral medication taken within 48 h was also recorded for all HF medications, including the amount of diuretics. Baseline physical findings and blood samples were taken and evaluated in the ED for all patients. Echocardiography was performed at the ED and subsequent steady-state phases. Left ventricular ejection fraction was assessed by echocardiography at the ED and categorized into 3 groups: <35%; 35% to 50%; and >50%. Importantly, the nature of this study did not require participants to use any particular drug or treatment strategy. The mission of the registry was to capture patients with AHF immediately after arrival to the ED and for the study enrollment process to avoid any delay or difference in the time course. Since obtaining written informed consent

at the ED may cause a delay in the ED management timeline, and subsequently biasing the results, we utilized an opt-out method for participant recruitment. All participants were notified regarding their participation in the study and it was explained that they were free to opt out of participation at any time. Our study complied with the Declaration of Helsinki and Japanese Ethical Guideline for Medical and Health Research involving Human Subjects. The study protocol was approved by the ethics committee of each participating hospital. Study information including objectives, inclusion and exclusion criteria, and the names of participating hospitals were published in the publically available University Hospital Information Network (UMIN-CTR, unique identifier: UMIN000014105) before the first patient was enrolled.

DOOR-TO-FUROSEMIDE TIME. Patients who were treated with IV furosemide within 48 h of ED arrival were included in the present study. The exact time of the first IV furosemide administration was recorded; the time from ED arrival to the administration of the first IV furosemide was defined as the door-to-furosemide (D2F) time. Patients with missing D2F time were excluded. To distinguish IV diuretics used for treating the initial AHF symptoms from treatment for deteriorated HF symptoms caused after the initial phase, we included only patients with a D2F time <24 h. In accordance with published recommendations of the European Society of Cardiology regarding early and prehospital management of AHF, we defined early and nonearly treatment groups using the D2F time with a cut-off of 60 min (8). The primary endpoint was all-cause in-hospital mortality.

STATISTICAL ANALYSIS

Data are expressed as mean ± SD for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed data. Categorical data are expressed as numbers and percentages. When necessary, variables were transformed for further analyses. Group differences were evaluated using Student t tests or Mann-Whitney U tests for continuous variables and chi-square or Fisher exact tests for categorical variables. The Get With the Guidelines-Heart Failure (GWTG-HF) risk score was calculated for each patient as previously described (10). The GWTG-HF risk score is based on race, age, systolic blood pressure, heart rate, blood urea nitrogen, sodium levels, and the presence of chronic obstructive pulmonary disease. The discrimination and calibration of this risk score have been well validated in Japanese patients with AHF (11); therefore, the GWTG-HF score was used as an adjustment variable in a multivariable prognostic model. Additionally, the patients were stratified according to GWTG-HF risk score quartiles and quartile groups were evaluated for differences in in-hospital mortality to examine the relationship between GWTG-HF scores at baseline and the prognostic impact of early treatment.

Univariable and multivariable logistic regression analyses were performed to evaluate the association between early treatment and in-hospital prognosis. Furthermore, generalized estimating equation (GEE) models were used to account for intrainstitutional correlations among patients. The presence of a nonlinear association between D2F time and in-hospital mortality was evaluated using a linear regression model with restricted cubic splines of 3, 4, and 5 knots. The goodness-of-fit was compared between models using an analysis of variance test.

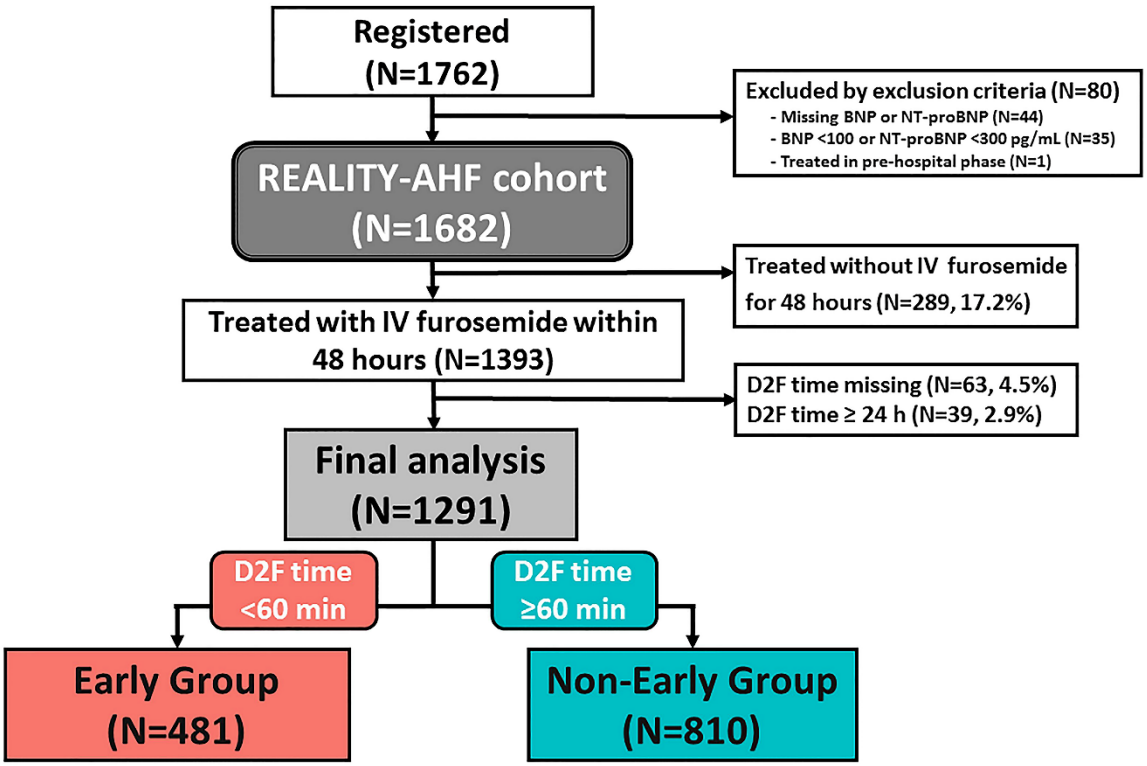
To control confounding as much as possible, propensity score matching was also performed as a sensitivity analysis. The propensity score was estimated based on a logistic model constructed with the following variables: age; sex; arrival by ambulance; baseline systolic blood pressure, diastolic blood pressure, heart rate, white blood cell count,

hemoglobin, aspartate aminotransferase, alanine aminotransferase, serum sodium, blood urea nitrogen, BNP, and glucose; history of chronic obstructive pulmonary disease; presence/absence of jugular venous distension, orthopnea, peripheral edema, or rales at baseline; and prescription of an angiotensin-converting enzyme inhibitor or loop diuretics at admission. Propensity score matching was performed for the early and nonearly treatment groups with one-to-one caliper matching using a caliper width equal to 20% of the SD of the logit of the calculated propensity score (12). To assess the performance of the matching, standardized mean differences were calculated for all baseline variables and a difference below 0.1 was considered negligible (i.e., the 2 groups were well balanced) (13).

A two-tailed p value < 0.05 was considered statistically significant. Statistical analyses were performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

During the study period, 1,762 patients meeting inclusion criteria were registered in the REALITY-AHF study (Figure 1). Eighty patients met exclusion criteria, leaving 1,682 patients; among these patients, 1,393 (82.8%) were given IV furosemide within 48 h of ED arrival. After excluding patients without an available D2F time or a D2F time of >24 h, the final cohort for analysis included 1,291 patients.



D2F time was non-normally distributed with a median value of 90 min (IQR: 36 to 186 min). Using a cutoff of 60 min, 481 patients (37.3%) were classified as the early treatment group and 810 (62.7%) comprised the nonearly

treatment group. Baseline characteristics for the groups are shown in Table 1. Patients in the early treatment group were more likely to arrive by ambulance; had acute onset of symptoms, higher blood pressure, and heart rate; and were more likely in sinus rhythm compared to those in the nonearly treatment group. Moreover, the early treatment group showed more signs of congestion, including higher New York Heart Association class and physical symptoms compared to the nonearly treatment group. Prescription of loop diuretics and mineralocorticoid receptor antagonists before admission were more prevalent in the nonearly compared to the early treatment group.

Table 1. Baseline Characteristics

Variables	Missing Data, n (%)	Early Treatment Group (n = 481)	Nonearly Treatment Group (n = 810)	p Value
Age, yrs	0	79 ± 11	78 ± 13	0.391
Male	0	254 (53)	471 (58)	0.072
Arrived by ambulance	0	349 (73)	428 (53)	<0.001
Symptom onset time	0			<0.001
≤6 h		150 (31)	162 (20)	
6 h-2 days		103 (21)	179 (22)	
> 2 days		228 (47)	469 (58)	
Systolic blood pressure, mm Hg	1 (0.1)	157 ± 37	147 ± 35	<0.001
Diastolic blood pressure, mm Hg	4 (0.3)	87 ± 26	83 ± 25	0.003
Heart rate, beats/min	0			
Total		102 ± 29	97 ± 28	0.001
Sinus patients		99 ± 22	93 ± 22	<0.001
AF patients		113 ± 32	105 ± 31	0.008
ECG rhythm	1 (0.1)			0.028
Sinus		286 (60)	419 (52)	
AF		155 (32)	307 (38)	
Others		40 (8)	83 (10)	
LVEF at ED	1 (0.1)			0.428
<35%		162 (37)	282 (37)	
35%-50%		144 (33)	222 (29)	
>50%		135 (30)	253 (34)	
NYHA III/IV at admission	1 (0.1)	426 (90)	632 (85)	0.012
Medical history				
History of heart failure	0	225 (47)	428 (53)	0.038

Hypertension	0	342 (71)	539 (67)	0.095
Diabetes mellitus	0	180 (37)	294 (36)	0.720
Coronary artery disease	0	160 (33)	240 (30)	0.191
COPD	0	52 (11)	74 (9)	0.333
Current or ex-smoker	0	191 (40)	305 (38)	0.478
Physical findings at ED				
Peripheral edema	1 (0.1)	366 (76)	547 (68)	0.001
JVD	21 (1.6)	326 (69)	444 (56)	<0.001
Orthopnea	4 (0.3)	361 (75)	453 (56)	<0.001
Pulmonary edema	0	391 (81)	586 (72)	<0.001
Rale	2 (0.2)	370 (77)	522 (65)	<0.001
Clinical profiles at ED	77 (6)			0.616
Warm-Dry		12 (3)	28 (4)	
Warm-Wet		391 (83)	594 (80)	
Cold-Dry		54 (11)	92 (12)	
Cold-Wet		16 (3)	27 (4)	
Medication at admission				
Loop diuretics	0	204 (42)	437 (54)	<0.001
ACE-I	5 (0.4)	78 (16)	141 (18)	0.592
ARB	4 (0.3)	159 (33)	243 (30)	0.291
Beta-blocker	8 (0.6)	190 (40)	341 (42)	0.379
Aldosterone blocker	1 (0.1)	86 (18)	192 (24)	0.014
Lab data				
WBC, /μl	0	8,200 (6,200–10,800)	7,400 (5,600–10,000)	<0.001
Hemoglobin, g/dl	0	11.5 (10.2-13.2)	11.7 (10.3-13.3)	0.512
AST, IU/l	1 (0.1)	32 (24-49)	31 (23-46)	0.833
ALT, IU/l	2 (0.2)	23 (14-35)	22 (14-36)	0.259
Creatinine, mg/dl	0	1.3 ± 0.9	1.4 ± 1.0	0.240
BUN, mg/dl	0	25 (18-33)	24 (18-35)	0.740
Glucose, mg/dl	51 (3.9)	181 ± 82	159 ± 73	<0.001
Sodium, mEq/l	1 (0.1)	139 ± 4	138 ± 5	0.452
CRP, mg/dl	3 (4)	0.64 (0.20-2.20)	0.73 (0.25-2.27)	0.396
BNP, pg/ml	126 (9.8)	737 (444-1,333)	746 (438-1,395)	0.836
Length of hospital stay, days	0	17 (11-26_	17 (11-26	0.457
GWTG-HF risk score	1 (0.1)	37 ± 8	38 ± 8	0.028

Values are mean ± SD, n (%), or median (interquartile range).
ACE-I = angiotensin-converting enzyme inhibitor; ALT = aspartate aminotransferase; ARB = angiotensin II receptor blocker; AST = alanine aminotransferase; BNP = B-type natriuretic peptide; BUN = blood urea nitrogen; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; ECG = electrocardiogram; ED = emergency department; GWTG-HF = Get With the Guidelines-Heart Failure; JVD = jugular venous distention; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; WBC = white blood cell.

Significant group differences in baseline laboratory markers were only found for the white blood cell count and glucose level. GWTG-HF risk score showed that the risk of the early treatment group was slightly lower than the nonearly treatment group. In terms of other treatments performed within 48 h, there were no significant group differences in the percentage of patients receiving catecholamines (dopamine, norepinephrine, or dobutamine): 16% in the early treatment group versus 15.5% in the nonearly treatment group (p = 0.812) or in the use of vasodilators (60.5% vs. 59.3%, respectively; p = 0.681). As more patients in the nonearly treatment group had a history of HF and had received treatment with furosemide before admission, we separately evaluated group differences in the amount of oral diuretics taken within 48 h among patients with and without a history of HF. No significant group differences in the amount of diuretics taken during this time period were found among those with (40 mg [IQR: 0 to 80 mg] vs. 40 mg [IQR: 20 to 80 mg]; p = 0.124) and without (0 mg [IQR: 0 to 30 mg] vs. 0 mg [IQR: 0 to 40 mg]; p = 0.690) a history of HF.

The results for the univariable and multivariable linear regression analyses predicting log-transformed D2F time are shown in Online Table 1 and Table 2, respectively. Arriving by ambulance and the presence of signs and symptoms of congestion, including orthopnea, jugular venous distension, angiotensin II receptor blocker prescription at admission, and a high heart rate were independently associated with a shorter D2F time. In the final multivariable linear regression model, none of the individual covariate variance inflation factors was greater than 2 and the mean variance inflation factor across all covariates was 1.06.

Table 2. Log-transformed Door-to-furosemide Time

Variable	B Coefficient*	Standardized* Beta	t Value*	p Value
Orthopnea	-0.455	-0.178	-6.278	<0.001
Arrived by ambulance	-0.323	-0.128	-4.646	<0.001
JVD	-0.307	-0.121	-4.371	<0.001
Heart rate	-0.003	-0.077	-2.781	0.006
Male	0.137	0.055	2.055	0.040
ARB at admission	-0.143	-0.054	-1.979	0.048

*A factor with a negative coefficient is associated with an earlier administration of intravenous furosemide.
Abbreviations as in Table 1.

D2F TIME AND IN-HOSPITAL MORTALITY

No patient was lost to follow-up for in-hospital outcome. During the index hospitalization, 11 patients (2.3%) in the early treatment group and 49 (6%) in the nonearly treatment group died (p = 0.002) (Figure 2). Although the

mortality rate increased as GWTG-HF risk score increased in both groups (p for trend < 0.05 for both groups), a lower mortality rate in the early treatment group compared to that for the nonearly treatment group was consistently observed across all quartiles, with the absolute risk difference increasing as GWTG-HF risk score quartile rose (p for trend = 0.027) (Figure 2). Table 3 shows the results of the univariable and multivariable logistic regression analyses. In logistic regression models, early treatment was associated with lower in-hospital mortality compared to nonearly treatment, even after adjustment for the GWTG-HF risk score. In GEE models, early treatment was associated with lower in-hospital mortality in univariate analysis (odds ratio [OR]: 0.36; 95% confidence interval [CI]: 0.21 to 0.62; p < 0.001) and after adjustment for the GWTG-HF risk score (OR: 0.42; 95% CI: 0.24 to 0.72; p < 0.001). In multivariable analysis, no significant interaction was seen between the early treatment group and GWTG-HF risk score on in-hospital mortality (p for interaction = 0.916). Additionally, there were no significant interactions between early treatment and any of the congestion symptoms (peripheral edema, jugular venous distension, orthopnea, pulmonary edema, and rales), arriving with ambulance or not, and sex (p for interaction > 0.3 for all). The association between early treatment and in-hospital mortality did not differ significantly in patients with a history of HF (OR: 0.43; 95% CI: 0.19 to 1.00; p = 0.051) and without a history of HF (OR: 0.34; 95% CI: 0.11 to 1.03; p = 0.057) (p for interaction = 0.738).

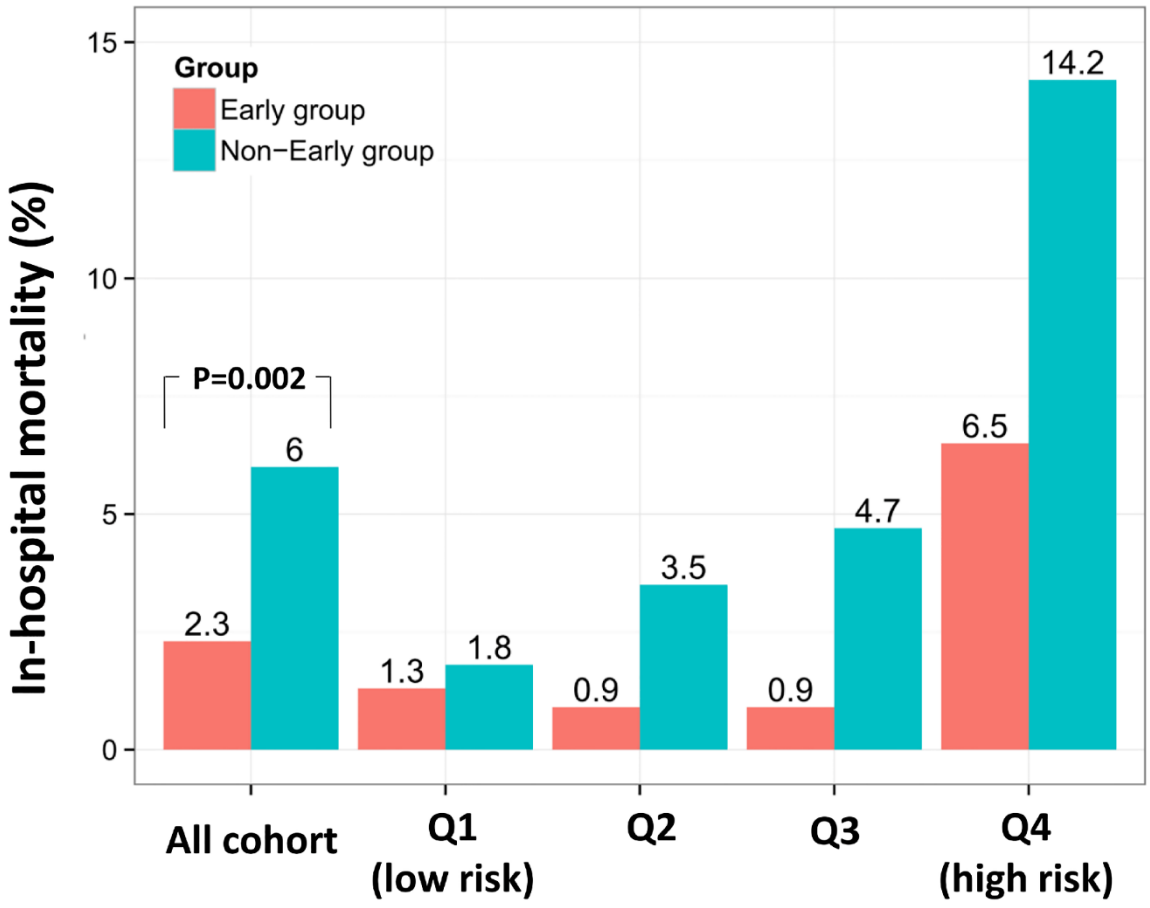


Figure 2. In-hospital Mortality
Early treatment resulted in lower mortality overall and stratified by quartiles of Get with the Guidelines-Heart Failure Score.

Table 3 Logistic Regression Analysis*

Adjustment	In-hospital Mortality			30-day Mortality		
	Odds Ratio	95% CI	p Value	Odds Ratio	95% CI	p Value
Univariate	0.36	0.19-0.71	0.002	0.52	0.28-0.96	0.037
Adjusted for GWTG-HF risk score	0.39	0.20-0.76	0.006	0.56	0.13-1.0	0.072
Propensity score matching	0.41	0.18-0.89	0.030	0.49	0.22-1.05	0.067

*Table includes univariate and multivariable logistic regression models for in-hospital mortality comparing the early to the nonearly treatment group.
CI = confidence interval; other abbreviations as in Table 1.

As a sensitivity analysis, we constructed a logistic regression model and GEE model using D2F time as a continuous scale (log D2F time) with an adjustment for GWTG-HF risk score. In both models, the association between log D2F time and in-hospital mortality remained statistically significant (OR: 1.31; 95% CI: 1.04 to 1.64; p = 0.021 for the logistic regression model and OR: 1.31; 95% CI: 1.01 to 1.71; p = 0.045 for the GEE model). In the propensity score analysis, 708 patients were matched based on the propensity score. The baseline characteristics of the cohort after matching are shown in Online Table 2. Among the matched patients, in-hospital mortality was, once again, lower in the early treatment group compared to that in the nonearly treatment group (5.9% vs. 2.5%; p = 0.038; OR: 0.41 [0.18 to 0.89]; p value = 0.030). Furthermore, we tested the association between early treatment and 30-day mortality from the index hospitalization, as there was variability in the length of hospital stay. In this additional sensitivity analysis, early treatment was associated with a lower 30-day mortality; however, the p value did not reach statistical significance in multivariable logistic regression analysis after adjusting for the GWTG-HF risk score or in the propensity score matching analysis (Table 3).

Figure 3 shows the association between D2F time and the probability of in-hospital mortality. Restricted cubic spline modeling with 4 knots was used as this model showed a better goodness-of-fit compared to that for the linear model and restricted 3-knot cubic spline model (p = 0.003 and p = 0.002, respectively), and showed a comparable goodness-of-fit to the 5-knot model (p = 0.579). The association between D2F time and predicted in-hospital mortality was not linear, and predicted mortality steeply increased in the first approximately 100 min from ED arrival and leveled off afterwards.

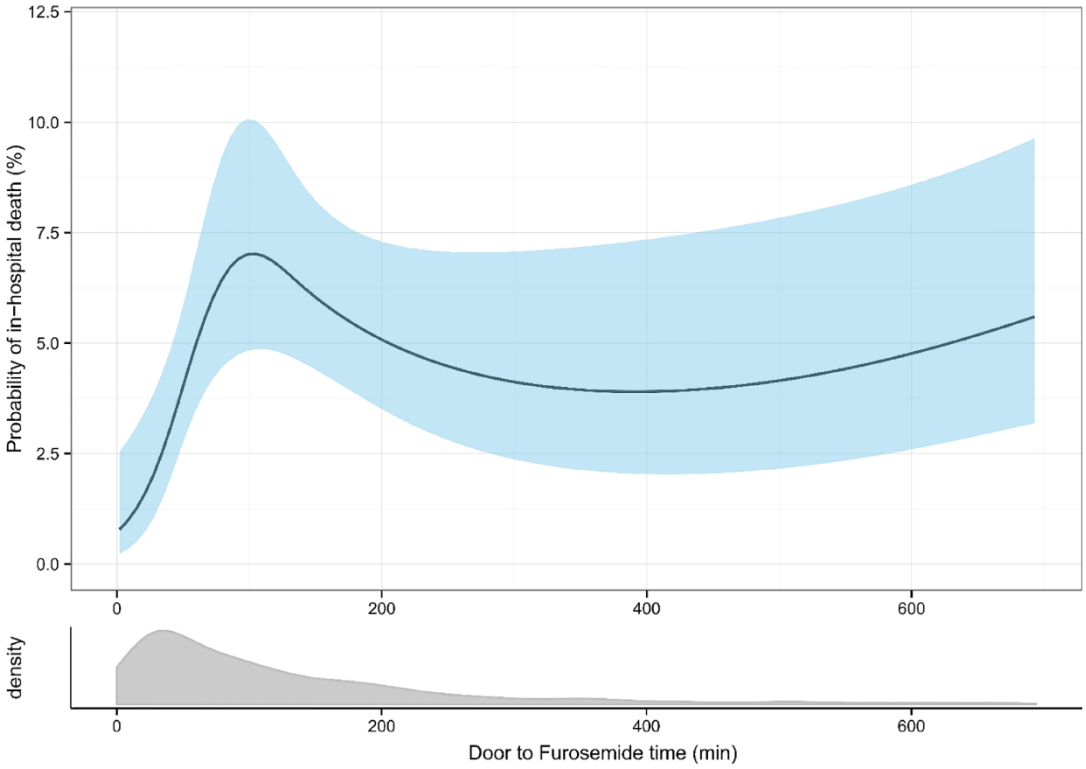


Figure 3. Probability Plot for In-hospital Mortality
The solid black line represents the estimated probability of in-hospital mortality, light blue shaded area is 95% confidence interval, and grey shaded area is a density plot showing the distribution of observed door-to-furosemide time.

DISCUSSION

A D2F time <60 min was observed in only one-third of patients with AHF who were hospitalized through the ED and treated with IV furosemide within 24 h. Patients with AHF presenting with more symptoms of congestion were more likely to be treated early. The association between D2F time and predicted in-hospital mortality had an inflection point; delaying D2F time steeply increased the mortality risk to the first approximately 100 min, but this effect leveled off thereafter (Central Illustration). D2F time <60 min was independently associated with a better in-hospital outcome.

D2F TIME AND PROGNOSTIC IMPLICATIONS

The present study showed that patients treated early were more likely to arrive by ambulance and had more prominent symptoms of congestion, consistent with previous studies performed in the ED setting (5,14). It is not surprising that the delivery of AHF treatment tended to be earlier in patients with more severe symptoms. Patients with symptoms and signs that were less obvious might have received the appropriate diagnosis at a later time point, and it is well known that the prompt and correct diagnosis of AHF in the ED remains challenging, especially for

patients without a typical sign of congestion, as no historical or physical examination finding with adequately high sensitivity and specificity exists (15). Likewise, it is obvious that arrival by ambulance leads to early treatment given that these patients would be more symptomatic and/or caregivers more likely to start evaluating and treating them early from a logistics point of view. It should be noted that female patients tended to be treated earlier than male patients. A recent post hoc analysis focused on dyspnea relief in the RELAX-AHF (Relaxin for the Treatment of Acute Heart Failure) study showed that female AHF patients were more prone to dyspnea than men (16).

Several retrospective studies have previously evaluated the prognostic impact of early treatment in patients with AHF. In ADHERE (Acute Decompensated Heart Failure National Registry), early treatment with nesiritide started in the ED was associated with a shorter mean hospital stay and less risk for a prolonged hospital stay (>7 days), but not with in-hospital mortality (17). However, as this analysis only included patients who were treated with nesiritide and excluded those treated with other vasodilators, the number of patients evaluated was narrowed from 105,388 to 4,300 (4.1%); thus, the generalizability of these results to all patients with AHF is unclear. Moreover, the use of other intravenous drugs, such as IV diuretics, was not taken into account in ADHERE, despite the fact that more than 95% of the patients were treated with IV diuretics, predominantly furosemide.

In another study using ADHERE data, an association between the time to administration of the first vasoactive agent and in-hospital mortality was evaluated in patients who received an IV vasoactive agent (nesiritide, nitroglycerin, nitroprusside, dobutamine, dopamine, or milrinone) (6). In this study, a shorter time to the first vasoactive agent was associated with better in-hospital mortality. This might support the results of the present study; however, early treatment in the previous study was defined as the use of an IV vasoactive agent ≤6 h from hospitalization (not ED arrival). As the time to hospitalization varies widely because many factors are involved, it is difficult to draw a conclusion regarding the beneficial impact of early treatment from this study. Also, similar to the aforementioned ADHERE study, only 25% of all initially registered patients were treated with IV vasoactive agents and were included in the study. Thus, selection biases might have existed and the generalizability of the findings is unknown.

The ADHERE-EM (emergency module) dataset is a retrospective study evaluating patients with AHF who presented at the ED. The prognostic implication of the time from ED admission to the first IV HF therapy administration (loop diuretics, inotropes, or vasodilators, whichever was administered first) was evaluated in 6,971 patients with AHF age ≥65 years registered in this dataset (14). Results showed that a delay in treatment was independently associated with a modest but significant increase in the risk of in-hospital mortality when time to treatment was examined as a continuous variable. Likewise, Maisel et al. retrospectively evaluated the association between time to first IV furosemide and in-hospital mortality in patients with AHF hospitalized via the ED using ADHERE registry data (5). The authors demonstrated that in-hospital mortality increased by 2.1% per every 4 h of delay in the time to first IV furosemide.

In contrast to previous studies, REALITY-AHF was a prospective study, specifically designed to examine the association between time to treatment and clinical outcome. One of the novel and interesting findings obtained from the present study was that the association between D2F time and in-hospital mortality might not be linear. In the first few hours after ED arrival, mortality steeply increased as D2F time was delayed, but this effect leveled off

after approximately 100 min. These findings might support the currently recommended time window of 30 to 60 min after ED arrival for the initiation of management for patients with AHF (8). It might also suggest that if physicians are more confident on the definite diagnosis of heart failure (more HF signs and symptoms), treatment with loop diuretics is commenced earlier and might be associated with better outcomes. Furthermore, no interaction between the prognostic impact of early treatment and baseline risk was found, and this treatment strategy might be even more effective in high-risk patients with AHF. Nonetheless, the present study and analysis could not determine the optimal D2F time, which needs to be evaluated in a future study.

The present study was not designed to provide a clear explanation regarding the pathophysiological mechanism underlying the association between D2F time and in-hospital outcomes. However, a recent sub-study of RELAX-AHF evaluated biomarker trajectories and showed that some organ damage markers (including high-sensitivity troponin) increased over time in the first 2 days in placebo groups and this increase was associated with mortality (18). Moreover, early treatment with serelaxin significantly attenuated the increase in high-sensitivity troponin within 2 days and decreased mortality. These results imply that myocardial damage is a progressive phenomenon in the acute phase among patients with AHF, and that early treatment mitigating this organ damage might consequently improve outcomes. Since we did not collect serial troponin data in the present study, this hypothesis should be evaluated in a future study.

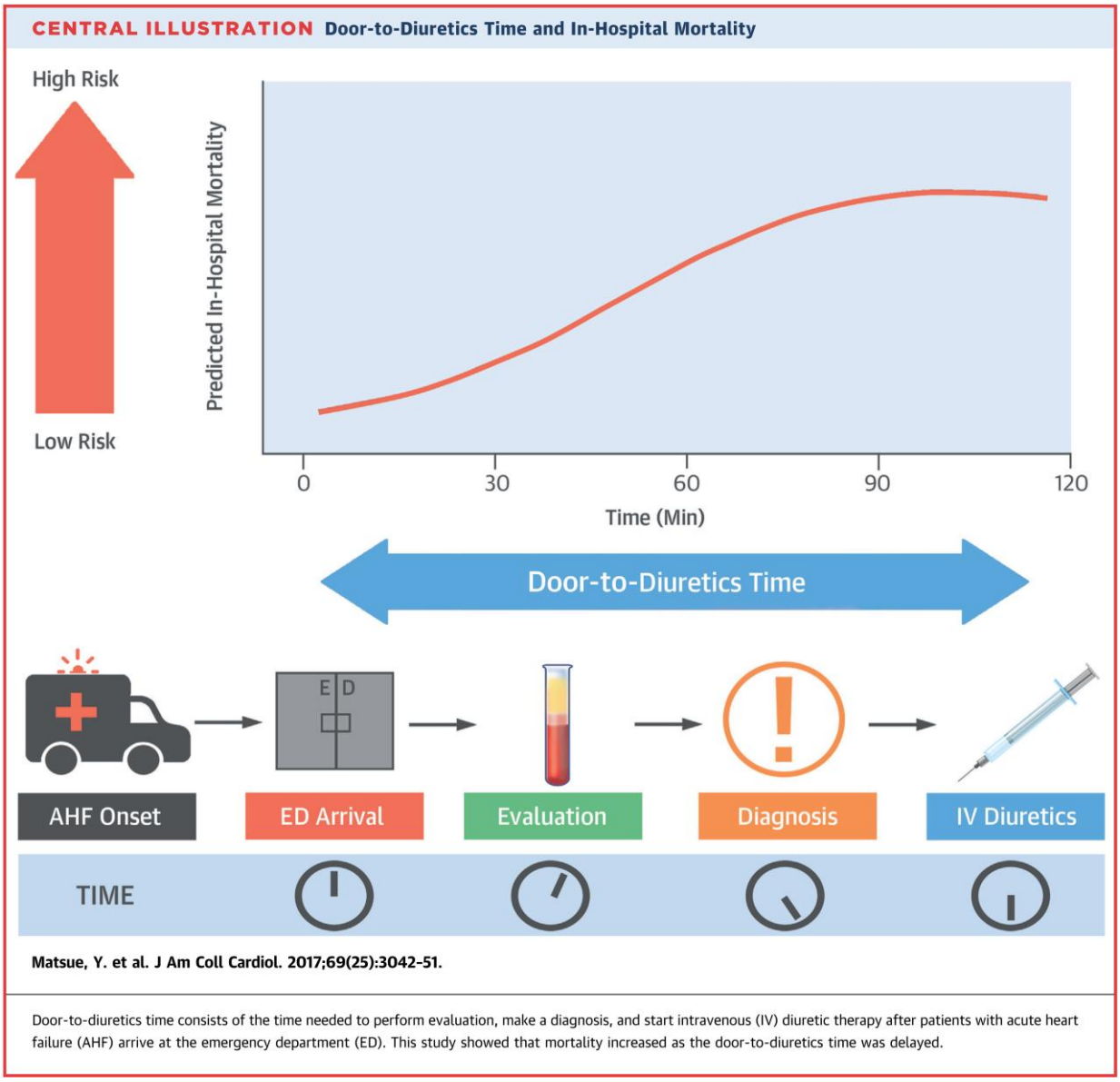
The present study findings may have an implication on both clinical practice and future clinical studies for patients with AHF. Previous major AHF clinical studies have not paid much attention to time to treatment and they consequently failed to recruit patients in the early phase of AHF (1). Although TRUE-AHF (Trial of Ularitide Efficacy and Safety in Acute Heart Failure) did not show positive results (19), despite recruitment of patients in the relatively early phase, the time from presentation to initiation of treatment appeared to be one of the most important factors to take into account in future clinical studies on AHF.

STUDY LIMITATIONS. All previous analyses regarding an association between early treatment and prognosis have been derived from only 1 dataset (ADHERE), which retrospectively registered hospitalized patients with HF without a focus on the time-to-treatment concept. The present study, for the first time, prospectively focused on this concept in patients with AHF. Given that we used D2F time as a metric of early treatment, our study results have wide applicability for patients with AHF, as a substantial proportion require IV furosemide in the acute phase.

There are several potential limitations that should be acknowledged. The small number of events in our study might lead to the risk of type I error and study results need to be replicated in future studies. We did not collect data regarding time to laboratory data or time to BNP or NT-proBNP measurement; thus, it is difficult to discriminate between time to diagnosis and time to treatment. Therefore, even with these data, it remains difficult to clearly define the exact time of diagnosis. We do not have data on the respiratory rate or cause of HF exacerbation, which might have an impact on outcomes. Also, an association between D2F time and long-term prognosis should be evaluated in a future study. In Japan, the median length of hospital stay is substantially longer than that for Western countries, which might affect the study results, especially for in-hospital mortality. However, in-hospital mortality was not substantially different between our study cohort and other registries representing American and European AHF patients (4.0% in ADHERE and 6.7% in EuroHeart Survey II) (20,21). Moreover, we obtained a similar association

between early treatment and 30-day mortality from the time of the index admission. As differences in ED systems potentially exist between institutions, our study results might not apply to all institutions. We attempted to adjust for confounders as much as possible by using a risk score that has been validated in Japanese patients with AHF, and showed consistency of our results in sensitivity analyses. Nevertheless, we agree with the possibility that a potential unadjusted confounding could remain, such as a “confounding by indication.” Therefore, our study results should be interpreted carefully as there is a considerable possibility that residual confounders remain since there were many differences in patient background between the early and nonearly groups. It should also be noted that D2F time may be associated with prognosis through its association with interinstitutional differences in quality of care for patients with AHF in the ED rather than any direct association with prognosis, although we showed a consistent result in the model accounting for it. Moreover, given its observational nature, it should be noted that only an association, not causality, was demonstrated in the present study.

These limitations clearly indicate that our study results are only hypothesis-generating; however, it is virtually impossible to randomize patients to a delayed-treatment group from an ethical point of view. As we included only patients hospitalized through the ED, the applicability of our study results to nonhospitalized patients is unclear. Additionally, we included only patients who were treated with IV furosemide within 24 h without any scientific basis for this time limit. However, recent studies regarding in-hospital worsening HF used 24 h from admission as the end of the acute phase (22,23). Moreover, changing the time-window for patient inclusion from 24 h to 48 h did not change our results substantially (data not shown).



CONCLUSIONS

In a prospective observational study focused on the acute phase in the management of patients with AHF, we demonstrated that patients with AHF and prominent congestive symptoms were more likely to be treated early with IV furosemide. Furthermore, treatment with IV furosemide within 60 min was independently associated with better in-hospital survival.

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Supplemental materials

Supplemental Table 1. Univariate logistic regression for early treatment

Variables	B coefficient	Standardized Beta	t value	P value
Age (years)	-0.002	-0.021	-0.746	0.456
Male gender (%)	0.121	0.049	1.761	0.078
Arrived by ambulance (%)	-0.429	-0.171	-6.220	<0.001
Symptom onset time				
≤ 6 hours		Reference		
6 hours - 2 days	0.337	0.113	3.363	<0.001
> 2 days	0.377	0.153	4.530	<0.001
Systolic blood pressure (mmHg)	-0.005	-0.144	-5.226	<0.001
Diastolic blood pressure (mmHg)	-0.005	-0.101	-3.631	<0.001
Heart rate (bpm)	-0.005	-0.117	-4.239	<0.001
Electrocardiogram rhythm (%)				
Sinus		Reference		
Atrial fibrillation	0.01	0.037	1.300	0.194
Others	0.186	0.044	1.546	0.122
LVEF at ED (%)				
<35%		Reference		
35-50%	-0.072	-0.027	-0.868	0.385
>50%	0.124	0.046	1.514	0.130
NYHA III/IV	-0.272	-0.077	-2.682	0.007
Medical History (%)				
Heart Failure	0.122	0.050	1.781	0.075
Hypertension	-0.089	-0.034	-1.209	0.227
Diabetes mellitus	-0.001	0.000	-0.016	0.987
Coronary artery disease	-0.064	-0.024	-0.863	0.388
COPD	-0.186	-0.045	-1.616	0.106
Current or ex-smoker	-0.096	-0.038	-1.368	0.172
Physical Examination at ED (%)				
Peripheral edema	-0.248	-0.092	-3.305	<0.001
JVD	-0.441	-0.174	-6.305	<0.001
Orthopnea	-0.613	-0.240	-8.874	<0.001
Pulmonary edema	-0.398	-0.139	-5.034	<0.001
Rale	-0.411	-0.154	-5.600	<0.001

Clinical profiles at ED (%)

Warm - Wet	Reference			
Warm - Dry	0.298	0.044	1.533	0.125
Cold - Dry	0.096	0.026	0.895	0.371
Cold- Wet	0.127	0.020	0.678	0.498
Medication				
Loop diuretics	0.2	0.081	2.926	0.003
ACE-I	-0.05	-0.015	-0.543	0.587
ARB	-0.137	-0.137	-1.859	0.063
Beta blocker	0.147	0.060	2.110	0.035
Aldosterone blocker	0.188	0.063	2.256	0.024
Laboratory data measured at ED				
WBC ($x10^6$/L)	-2.327	-0.069	-2.494	0.013
Hemoglobin (g/dL)	-0.004	0.008	-0.299	0.765
ALT (IU/L)	0.00003	0.003	0.104	0.917
AST (IU/L)	0.00005	0.005	0.164	0.869
Creatinine (mg/dL)	0.016	0.012	0.447	0.655
BUN (mg/dL)	0.002	0.023	0.811	0.417
Glucose (mg/dL)	-0.002	-0.123	-4.349	<0.001
Sodium (mEq/L)	-0.01	-0.039	-1.411	0.159
CRP (mg/dL)	0.011	0.030	1.063	0.288
BNP (pg/mL)	0.00005	0.047	1.607	0.108

ACE-I, angiotensin converting enzyme inhibitor; ALT, aspartate aminotransferase; ARB, angiotensin II receptor blocker; AST, alanine aminotransferase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ED, emergency department; GWTG-HF, Get With the Guideline Heart Failure; JVD, jugular venous distention; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association

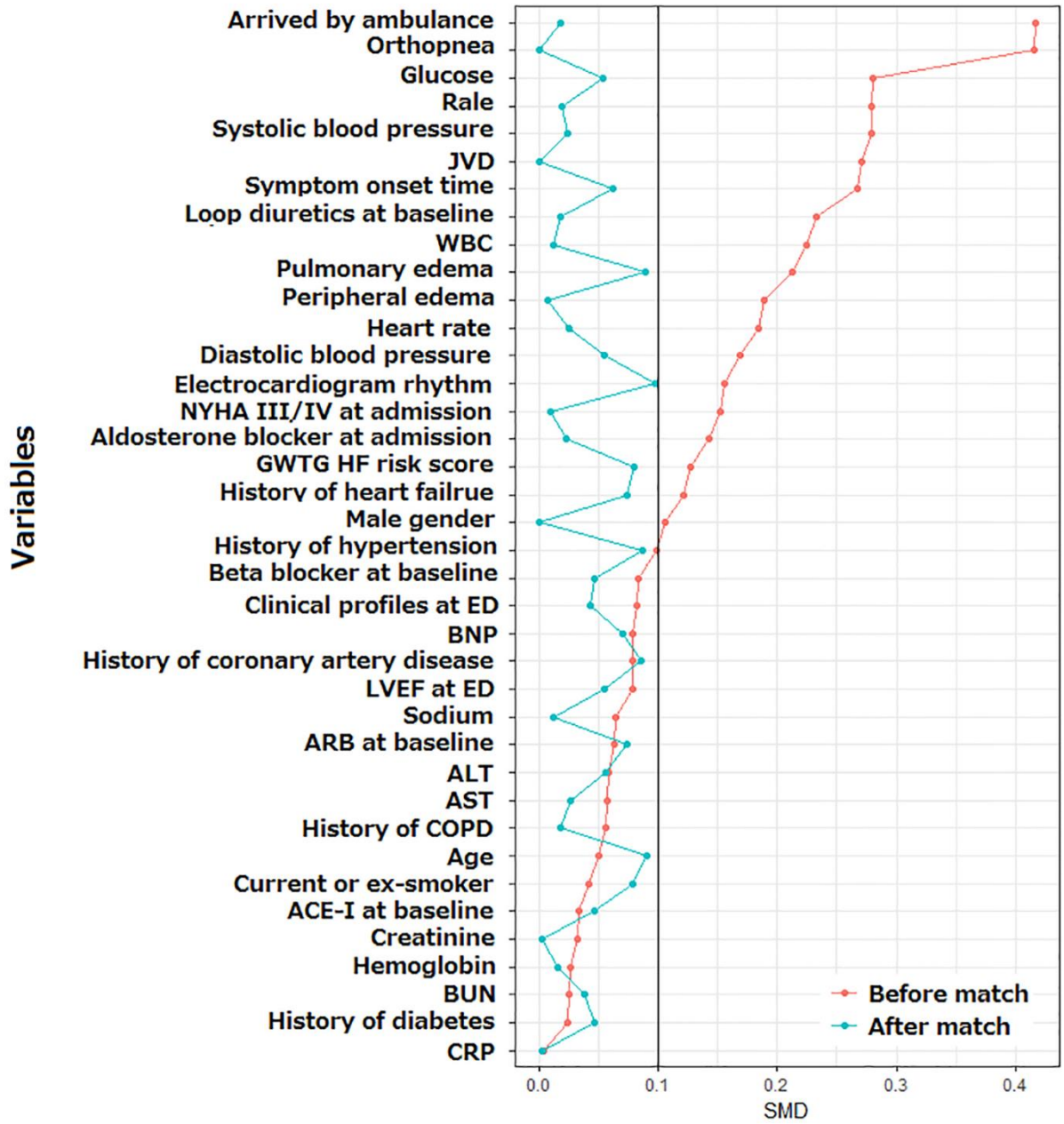
Supplemental Table 2. Baseline characteristics before and after propensity score matching

Variables	Before match		After match	
	Early treatment group (n=481)	Non-early treatment group (n=810)	Early treatment group (n=354)	Non-early treatment group (n=354)
Age (years)	79±11	78±13	78±11	77±13
Male gender (%)	254 (53)	471 (58)	201 (57)	201 (57)
Arrived by ambulance (%)	349 (73)	428 (53)	241 (68)	238 (67)
Symptom onset time				
≤ 6 hours	150 (31)	162 (20)	98 (28)	96 (27)
6 hours - 2 days	103 (21)	179 (22)	75 (21)	84 (24)
> 2 days	228 (47)	469 (58)	181 (51)	174 (49)
Systolic blood pressure (mmHg)	157±37	147±35	156±38	157±37
Diastolic blood pressure (mmHg)	87±26	83±25	88±26	89±27
Heart rate (bpm)	102±29	97±28	101±29	102±27
ECG rhythm (%)				
Sinus	286 (60)	419 (52)	198 (56)	191 (54)
AF	155 (32)	307 (38)	121 (34)	135 (38)
Others	40 (8)	83 (10)	35 (10)	28 (8)
LVEF at ED (%)				
<35%	162 (37)	282 (37)	121 (36)	119 (36)
35-50%	144 (33)	222 (29)	108 (32)	102 (31)
>50%	135 (30)	253 (34)	104 (31)	112 (34)
NYHA III/IV at admission (%)	426 (90)	632 (85)	315 (89)	316 (89)
Medical history (%)				
History of Heart Failure	225 (47)	428 (53)	176 (50)	163 (46)
Hypertension	342 (71)	539 (67)	253 (72)	239 (68)
Diabetes mellitus	180 (37)	294 (36)	132 (37)	140 (40)
Coronary artery disease	160 (33)	240 (30)	120 (34)	106 (30)
COPD	52 (11)	74 (9)	43 (12)	41 (12)
Current or ex-smoker	191 (40)	305 (38)	157 (44)	143 (41)
Physical findings at ED (%)				
Peripheral edema	366 (76)	547 (68)	255 (72)	256 (72)

JVD	326 (69)	444 (56)	232 (66)	232 (66)
Orthopnea	361 (75)	453 (56)	255 (72)	255 (72)
Pulmonary edema	391 (81)	586 (72)	285 (81)	297 (84)
Rale	370 (77)	522 (65)	261 (74)	264 (75)
Clinical profiles at ED (%)				
Warm - Dry	12 (3)	28 (4)	11 (3)	11 (3)
Warm - Wet	391 (83)	594 (80)	283 (80)	282 (80)
Cold - Dry	54 (11)	92 (12)	43 (12)	47 (13)
Cold- Wet	16 (3)	27 (4)	16 (5)	14 (4)
Medication at admission (%)				
Loop diuretics	204 (42)	437 (54)	163 (46)	160 (45)
ACE-I	78 (16)	141 (18)	54 (15)	60 (17)
ARB	159 (33)	243 (30)	114 (32)	102 (29)
Beta blocker	190 (40)	341 (42)	143 (40)	135 (38)
Aldosterone blocker	86 (18)	192 (24)	70 (20)	67 (19)
Lab data				
WBC (/µl)	8200 [6200 - 10800]	7400 [5600 - 10000]	8100 [6200 - 10700]	8400 [6025 - 10900]
Hemoglobin (g/dL)	11.5 [10.2 - 13.2]	11.7 [10.3 - 13.3]	11.6 [10.3 - 13.2]	11.8 [10.2 - 13.4]
AST (IU/L)	32 [24 - 49]	31 [23 - 46]	32 [23 - 47]	31 [24 - 45]
ALT (IU/L)	23 [14 - 35]	22 [14 - 36]	22 [14 - 34]	20 [14 - 33]
Creatinine (mg/dL)	1.3±0.9	1.4±1.0	1.3±0.8	1.3±0.9
BUN (mg/dL)	25 [18 - 33]	24 [18 - 35]	25 [18 - 33]	24 [17 - 33]
Glucose (mg/dL)	181±82	159±73	172±78	176±85
Sodium (mEq/L)	139±4	138±5	139±5	139±5
CRP (mg/dL)	0.64 [0.20 - 2.20]	0.73 [0.25 - 2.27]	0.74 [0.23 - 2.35]	0.66 [0.24 - 2.17]
BNP (pg/mL)	737 [444 - 1333]	746 [438 - 1395]	752 [449 - 1302]	719 [432 - 1255]
GWTG-HF risk score	37±8	38±8	37±8	37±8

ACE-I, angiotensin converting enzyme inhibitor; ALT, aspartate aminotransferase; ARB, angiotensin II receptor blocker; AST, alanine aminotransferase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ED, emergency department; GWTG-HF, Get With the Guideline Heart Failure; JVD, jugular venous distention; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association

Supplemental Figure 1. Standardized mean difference before and after propensity score matching



Standardized mean differences were below 0.1 for all baseline variables, including each component of the GWTG-HF risk score and score itself. The C-statistics of propensity for the logistic model used to generate propensity scores was 0.70 (95% CI: 0.66 – 0.73) and Hosmer-Lemeshow test was not significant (P = 0.20).

Chapter 8

Summary and future perspectives

General discussion

Acute heart failure (AHF) is a syndrome that not only involves the heart, but affects many other organs as well. The presence of these co-morbidities makes the pathophysiology, phenotype and treatment of this heterogeneous syndrome very complicated. Renal dysfunction is one of the most prevalent comorbidities with a strong association with clinical outcome, but a deeper understanding of the pathophysiology is lacking. Several studies that tested the effect of drug treatment in AHF with concomitant renal dysfunction have shown neutral results similar to other randomized clinical studies in patients with AHF. To better understand the pathophysiology behind renal dysfunction in patients with heart failure, a crucial step is to seek a successful intervention for this high-risk subgroup. A biomarker is a powerful tool, not only for predicting prognosis, but also for examining the biological pathway of the disease. Numerous studies on renal biomarkers have been conducted and most showed their strong association with prognosis. However, currently available renal biomarkers are not capable of providing information on precipitating factors of renal dysfunction in heart failure, which can vary from patient to patient. Therefore, attempting to use preexisting and novel biomarkers to derive such information regarding renal pathophysiology is clinically relevant for developing effective treatment for AHF patients, including those with concomitant renal dysfunction.

Treatment of patients with AHF has not changed significantly for several decades, and there is no approved drug to adequately improve prognosis. Even though AHF has been acknowledged as a life-threatening disease that requires treatment as quickly as possible, surprisingly few studies have investigated the appropriate timing of interventions in patients with AHF. A wide variety of contemporary randomized control trials on AHF have examined the timing of the initial intervention, yet most of them have failed to include participants in the early phase. Given there are some data supporting the idea of “the earlier, the better,” it is vital to obtain more data and insights into this area from patient care and scientific perspectives.

The primary aims of this thesis were:

- To position the potentially novel and conventional renal biomarkers
- To test the effectiveness of early treatment with aquaretics in AHF patients with concomitant renal dysfunction
- To investigate the prognostic implication of early treatment with diuretics in patients with AHF

In Chapter 2, we positioned a novel biomarker, pro-enkephalin (pro-ENK), in patient with heart failure. Pro-ENK is a precursor of enkephalin, which has been long-recognized and studied in the field of neuronal tissues. However, many studies showed (pro-) enkephalin is implicated in the cardiovascular regulation system, and one study showed that pro-ENK was a biomarker associated with renal function and prognosis in patients with acute myocardial infarction. Because the clinical value of this biomarker had not been evaluated in heart failure patients, we measured pro-ENK in 95 chronic heart failure patients (renal mechanistic cohort) whose glomerular function was

well-evaluated using ^{131}I-Hippuran and ^{125}I-Iothalamate to evaluate its association with renal function. In addition, to elucidate the prognostic predictability of pro-ENK in patients with AHF, the levels of pro-ENK were evaluated in 1589 patients in the PROTECT dataset (AHF cohort). In a renal mechanistic cohort, we found pro-ENK levels to be strongly associated with glomerular function and measured (not estimated) glomerular function using radioactive tracers was the most powerful predictor of pro-ENK levels. In contrast, pro-ENK was not associated with urinary tubular markers (NAG, NGAL, and KIM-1). In the AHF cohort, again, high pro-ENK levels were strongly associated with low glomerular markers (creatinine and blood urea nitrogen) and worse outcomes. However, its prognostic predictability was not retained after adjusting for pre-existing renal biomarkers, including creatinine and BUN. Altogether, we positioned pro-ENK as a glomerular marker rather than a tubular marker in patients with heart failure. Pro-ENK was associated with a worse prognosis in patients with AHF; however, its additive prognostic information to pre-existing renal markers is limited.

In Chapter 3, we attempted to define the normal range of the blood urea nitrogen (BUN)/creatinine ratio in the general population (PREVEND cohort), and the prognostic value of being out of normal range in patients with AHF. Like creatinine, BUN is a readily available renal biomarker in daily clinical practice. From a metabolic point of view, BUN is similar to creatinine in that it is freely filtrated at the glomerulus and, therefore, strongly associated with renal function, particularly with glomerular function. However, contrary to the fact that creatinine is not reabsorbed in tubules, approximately 40-50% of BUN is reabsorbed in the tubules and this process is directly and indirectly handled by neurohormonal activity including the renin-angiotensin-aldosterone system, sympathetic nerves, and arginine-vasopressin system. This difference forms the pathophysiological basis of the BUN/creatinine ratio as a metric of neurohormonal activity in patients with heart failure. There have been many studies that have showed the association between high BUN/creatinine ratio and poor prognosis in patients with heart failure, but no study has investigated the normal range of BUN/creatinine ratio. We sought to define the normal range of BUN/creatinine ratio using data derived from the general population without overt cardiovascular comorbidities and to find its prognostic meaning in patients with AHF. In the general population, we found that the BUN/creatinine ratio increased with age, and significantly more in females compared with males. Moreover, the BUN/creatinine ratio varied widely in the general population, which may imply that using a reference range might be useful to define patients with a high BUN/creatinine ratio. Patients with a higher than normal range BUN/creatinine ratio are associated with indices that indicate a highly activated neurohormonal system. A higher than normal range BUN/creatinine ratio among patients was associated with worse outcomes compared with patients within normal range, and this association remained even after adjusting for pre-existing renal biomarkers, including creatinine and BUN. This finding suggests that to know if BUN/creatinine ratio is out of normal range may provide us with pathophysiological information on the renal function in AHF patients that cannot be provided otherwise. However, this hypothesis should be tested in a study that has clearer pathophysiological information on the background of renal dysfunction in patients with AHF.

We tested the hypothesis that early treatment with tolvaptan might be effective in AHF patients with concomitant

renal dysfunction in Chapters 4, 5, and 6.

In Chapter 4, we described the rationale for our study. Among AHF patients, those with renal dysfunction are at especially high-risk of poor outcome. One of the clinical concerns of this subgroup is diuretic resistance, and subsequent unsatisfactory decongestion, which has shown to be strongly associated with a poor prognosis. Tolvaptan is an emerging new class of diuretics that causes electrolyte-free water excretion and yields urine output in a different form. Tolvaptan's clinical effectiveness was tested in a large-scale, double-blinded, randomized clinical trial, EVEREST, which revealed that tolvaptan was significantly more effective in causing decongestion in the short-term, but found no mid-term prognostic benefit compared to placebo. However, EVEREST did not focus on patients with renal dysfunction. More importantly, EVEREST enrolled participants relatively late after admission, similar to other contemporary AHF studies, which leads to Chapter 7 in which tolvaptan's clinical implications are more explicitly discussed. Therefore, we set out to test the hypothesis that early treatment, within 6 hours of admission, with tolvaptan can more effectively and safely treat AHF patients with renal dysfunction compared to conventional treatment. We designed a multicenter, open-label, randomized clinical trial, AQUAMARINE, to test this hypothesis and enrolled 220 Japanese AHF patients with renal dysfunction defined as an estimated GFR 15 to 60 mL/min/1.73 m^2. The primary endpoint was urine output achieved within 48 hours of randomization.

The main findings of the AQUAMARINE study are presented in Chapter 5. Two-hundred twenty AHF patients whose estimated GFR below 60 mL/min/1.73 m^2 were enrolled and randomized to either the tolvaptan treatment group or conventional treatment group. Patients in the tolvaptan group were treated with tolvaptan add-on therapy (15 mg add-on therapy once daily for 2 days). Median time to randomization from admission was 1 hour, and 41.4% of patients were randomized before admission at either the emergency department or clinic. After three patients were excluded, 217 patients were included in the analysis. The tolvaptan group yielded significantly more urine output compared to the conventional group (6464 mL vs. 4997 mL, P <0.001). Regarding secondary outcomes, we observed a significantly greater reduction in body weight, more frequent dyspnea relief at all time points within 48 hours, except 6 hours after randomization in favor of the tolvaptan group, even though less amount of furosemide was used in the tolvaptan group compared to the conventional group. On the other hand, incidence of worsening renal function (WRF), which was one of the prespecified secondary endpoints and defined as ≥0.3 mg/dL increase in creatinine from baseline, did not significantly differ between groups. Likewise, there was no significant difference in the trajectory of creatinine measured at several prespecified time points (6, 12, 24, and 48 hours of randomization). Furthermore, no differences were observed between the tolvaptan group and conventional group in incidence of any adverse events and combined endpoint of all-cause death and heart failure readmission within 90 days of randomization. Although our study results should be interpreted cautiously, due to its open-label study design, successful very early patients' enrollment/randomization and subsequent positive results in the primary endpoint might suggest that early use of tolvaptan could be an option for AHF patients with renal dysfunction.

In Chapter 6, we investigated the influence of early treatment of tolvaptan on the diuretic response using the

AQUAMARINE study dataset. In this analysis, diuretic responses were evaluated for each patient as the change in body weight from baseline to 48 hours and net fluid loss within 48 hours per 40 mg intravenous furosemide-equivalent dose. We found that patients with a poor diuretic response were less likely to have dyspnea relief within 48 hours and were more likely to experience WRF defined as a ≥0.3 mg/dL increase in creatinine from baseline. Tolvaptan was independently associated with a good diuretic response in both definitions. Also, we found that the diuretic response in the conventional groups was relatively better than our expectation given that only patients whose estimated GFR was below 60 mL/min/1.73 m^2 were included in AQUAMARINE study. There are several possible explanations for this finding, including early treatment contributes to a better diuretic response; however, this hypothesis needs to be evaluated in another study that is more focused on this topic. Together with Chapter 5, we showed the effectiveness of early treatment with tolvaptan in AHF patients with renal dysfunction in terms of decongestion. However, once again, these findings should be interpreted cautiously as our study tested a small sample size and, more importantly, in an open-label fashion.

Clinical implications of the concept “time to treatment” in patients with AHF was directly evaluated in Chapter 7. Although some studies have already suggested a favorable prognostic impact of early treatment in patients with AHF, all were retrospective studies using the same large, but retrospective registry dataset (ADHERE registry). We conducted a multicenter prospective registry, REALITY-AHF, primarily focusing on the relationship between early treatment and prognosis in AHF patients hospitalized through the emergency department with 20 participating institutions in Japan. We analyzed 1291 AHF patient who were treated with intravenous furosemide within 24 hours of emergency department arrival. Door-to-furosemide time was defined as the time from patient arrival at the emergency department to the first intravenous furosemide injection and prospectively collected. About one third of patients were treated within 60 minutes, and this was associated with lower in-hospital mortality compared to those who were treated later than 60 minutes with intravenous furosemide. Moreover, we found a non-linear association between door-to-furosemide time and in-hospital mortality; predicted in-hospital mortality steeply increased in the first approximately 100 minutes from patient arrival in the emergency department and leveled off afterwards. This prospectively obtained data give us, for the first time, a clear basis for advocating early treatment with diuretics in AHF patients who require decongestion from a prognostic point of view.

Future perspectives

Cardiorenal biomarkers in AHF

Renal dysfunction is one of the most common comorbidities in patients with AHF and its association with worse outcomes is robustly proven. Biomarker studies have played a pivotal role in the progress towards understanding this association. However, the understanding of the pathophysiological background of renal dysfunction in heart failure is far from satisfactory. There are several reasons which hamper progress in comprehending this complex relationship.

First, we do not have a renal biomarker capable of capturing renal function accurately and timely in patients with AHF. Conventionally, GFR has been used to explain the majority of renal function and has played the most important role. The gold standard method of measuring GFR is using plasma or urinary clearance of an exogenous filtration marker, such as inulin, or measuring with radioisotope or iothalamate methods. However, since these are realistically not available in daily clinical practice, estimated GFR from serum creatinine is generally used in the clinical practice setting to evaluate glomerular function. Several studies have validated the accuracy of creatinine-based GFR in the general population and some modified equations have been developed for better estimating GFR; however, few studies have validated this estimation method in patients with heart failure. In addition, it should be noted that no study has validated creatinine-based estimated GFR in patients with AHF. Given that creatinine is not a suitable biomarker, especially in a non-stable setting, as it is unable to reflect a rapidly changing GFR and starts elevating when at least at 50% of GFR loss; the accuracy of creatinine-based estimated GFR in patients with acute rather than chronic HF is unclear. Based on these reasons, creatinine might not be an ideal biomarker to evaluate glomerular function in patients with AHF.

Second, we do not have a renal biomarker that provides pathophysiological information on the cause of renal dysfunction in patients with heart failure. Currently, we are able to recognize only the consequence, but not the causes, of deteriorating renal function using biomarkers. This important limitation has been reinforced by many studies, which have shown that increased creatinine is not always translated into poor prognosis as once was thought. Clinical circumstances surrounding an increase in creatinine is important to understand if it is likely to accompany subsequent unfavorable outcomes. For instance, Metra et al. showed that WRF results in inadequate decongestion during hospitalization, but not that WRF with adequate decongestion leads to a worse prognosis compared to those without WRF. Also, WRF caused by administering an angiotensin converting enzyme inhibitor in patients with heart failure with reduced ejection fraction (HFrEF) is not associated with better outcomes. On the other hand, an increase in creatinine occurring after introducing an ARB was associated with worse outcomes in patients with heart failure who have a preserved ejection fraction (HFpEF). The difference in the prognostic impact of worsening renal function between HFrEF and HFpEF was validated in a recent meta-analysis. These “conditional” associations between WRF and prognosis suggest the importance of the cause of an increase in creatinine rather than the increase itself, and the phenotyping of WRF, which might be enabled with renal biomarkers capable of providing renal pathophysiological information.

Finally, we do not have a renal biomarker encompassing all functions of the whole kidney. Several studies have underscored the prognostic importance of tubular damage measured by several tubular markers (e.g., NGAL, KIM-1, and beta-2-microglobulin) in patients with acute heart failure. These tubular markers have prognostic predictive ability independent from glomerular markers. This implies we should integrate information on glomerular function with information on tubular damage to better comprehend renal function. It should also be noted that there are biomarkers available for evaluating tubular “dys”-function, but the definition and gold standard measurement of tubular function have yet to be determined. The renal biomarker, which is implicated in both tubular and

glomerular pathophysiology and allows us to comprehend the whole picture of renal function, will be welcome.

There is no doubt that biomarkers have been contributing to the diagnosis, risk stratification, and understanding of the underlying pathophysiology of heart failure. On the other hand, it is fair to say very few new and old biomarkers have changed our daily clinical practice when it comes to treatment/intervention as no clear answer has been shown to have clinical implications in biomarker-guided therapy trials. To better understand and manage AHF patients with concomitant renal dysfunction, it is essential to keep looking for the biomarker that describes much about the "kidney" and not the "prognosis" in patients with heart failure. To speak of extremes, we do not know if we can treat AHF patient better with biomarkers even compared to "zero-biomarker" treatment. In fact, most of the recommended acute-phase treatments for patients with AHF were based on physical findings or simple vital signs but not on biomarkers at the moment. We might also have to rethink of cost-effectiveness of utilizing biomarker in management of AHF patients as some biomarkers are costly.

Another general question that remains regarding biomarker research in heart failure is that of causality. Most previous studies that evaluated the significance of biomarkers have showed only an association rather than causality. Therefore the next critical step we need to take in the use and research on biomarkers is to consider the presence or absence of causality. From a biological point of view, each biomarker is implicated in several biological pathways and can be influenced by many factors which also impact outcomes. This makes it very difficult to test a pure association between biomarkers and outcomes of interest, even though many sophisticated statistical analyses are used to adjust for confounding factors. Recent progress in genetic analyses and genome-wide association studies now enable us to infer causality between biomarkers and outcomes, including prognosis. This process is quite important in many aspects. The biomarker can be a very strong surrogate marker for testing treatment strategies or the development of new drugs if the causality between the biomarker and the outcome is in the direction of biomarker to outcome. Considering some recent studies have shown the lack of (or very little, at least) causality between some biomarkers and outcomes which has been proven by many observational studies, a similar approach should also be taken for renal biomarkers in HF patients. Mendelian randomization, which is one of the means to allow us to reason causality, has been increasingly used in medical research; however, there is no study applying this novel, time- and cost-effective approach to the heart failure population. Narrowing down only the biomarkers that have a causal association with clinically relevant outcomes should aid us in accelerating biomarker research in the field of heart failure.

Optimal timing for treatment in AHF

Heart failure is a progressive disease, and symptoms change and fluctuate overtime. Even if the patient's condition has been stable for years, it can abruptly collapse and decompensate within a couple of minutes. Therefore, it is very natural to say that care providers need to provide the right treatment to the right patients at the right time; however, most of the AHF clinical randomized studies, which have been performed in the last decades, have paid attention mostly to the means of intervention and not much on timing and patient selection. The notion "the earlier the treatment, the better outcome" has been well-tested and accepted in acute coronary syndrome patient care;

several retrospective studies have shown the same association between time to treatment and several outcomes. Nonetheless, there has been no study focusing on this timing of treatment in AHF patients. In this thesis, we confirmed the favorable association between early treatment and outcomes in AHF patients, which encourages us to take, more seriously, into account this notion in both daily clinical practice and in designing clinical studies. To data, a few AHF randomized control trials successfully enrolled patients relatively early. In the RELAX-AHF, mean time from admission to randomization was 7.9 hours and serelaxin did not affect the composite of heart failure hospitalization or death through 60 days. Treatment with serelaxin was associated with lower 180 days all-cause and cardiovascular mortality; however, this finding was not replicated in RELAX-AHF-2 study. In the TRUE-AHF study, all patients were allocated to either ularitide or placebo in a median of 6.1 hours after the initial clinical evaluation and showed a neutral impact on prognosis. These results might contradict the idea we proposed; however, there are many alternative explanations. Both serelaxin and ularitide was shown to reduce blood pressure and BNP in the study period, but it does not necessarily mean its associated with a better prognosis. It should be noted that the neutral prognostic impact of this early treatment with these AHF drugs can be generalized providing that these are the drug capable of improving prognosis. However, thus far, no study has shown a favorable impact of any kind of vasodilator on prognosis, and it is fair to say the results of RELAX-AHF and TRUE-AHF did not fully address the hypothesis. In Chapter 7, we suggested that the possible optimal timing for starting treatment might be much earlier than 6 hours from the time of patient admission. This result supports our speculation proposed in Chapter 6; we inferred that one of the reasons for observing an unexpectedly good diuretic response in the AQUAMARINE cohort might be the early treatment provided (i.e., a median of 2.1 hours from the time of patient evaluation). Alternatively, these results raise additional questions, especially about the pathophysiological background of its beneficial effect and “optimal” timing for early intervention in patients with decompensated heart failure. One possible explanation for favorable prognostic impact of early treatment with diuretics is that early treatment might mitigate organ damage progressing in acute phase and consequently improve outcomes. However, at the same time, it should be noted that patients with early treatment showed significantly different patient characteristics compared to those without, and this difference could contribute to difference in prognosis. As the worsening and improving of heart failure symptoms are not clearly distinguishable statuses, and are rather sequential in nature, an approach that allows us to intervene with the patient that is about to become decompensated might be more optimal. Indeed, a recent study, the CHAMPION trial, using a pulmonary artery pressure monitoring device showed results compatible with our hypothesis. Overall, although the data on the timing of treatment in patients with acute heart failure consistently show a significant influence on outcomes, including prognosis, the optimal timing and means of intervention remain to be clarified in future studies.

In conclusion, renal biomarkers have helped us identify AHF patients at high risk; however, their utility in optimizing treatment for AHF patients with concomitant renal dysfunction has yet to be realized. To make this a reality, it is important to not only keep looking for a novel renal biomarker, but also to seek an effective incorporation of pre-existing renal biomarkers in clinical practice. The same is true for interventions in patients with AHF. Finding a better use of pre-existing drugs is as important as developing new drugs. In the last few decades, several newly developed

drugs have been examined in randomized clinical trials; however, most of the trials resulted in neutral results, yet much has been learned in the process. Hopefully, this thesis will serve to help researchers to consider how the problem can be tackled that we have been unable to successfully solve before.

Appendices

Acknowledgement

Dear Prof. Dr. Adriaan Voors, when you kindly accepted me as a research fellow in your heart failure research team in 2014, I was very grateful. Although no one introduced me to you and you did not know anything about me, you were open-minded enough to accept me. You are not only a great scientist and clinical researcher, but also an encouraging mentor for your students and colleagues. I could not have completed my Ph.D. project without your help. During my three-and-a-half-year stay in Groningen, I learned a lot about clinical research, but what I learned the most from you were the most important parts of clinical research. I will strive to remain in the heart failure clinical research field, and I am very grateful for your support.

Dear Prof. Dr. Peter van der Meer, you have been one of my supervisors from the beginning, and always gave me advice that guided me in the right direction. I loved reading your comments on my manuscript drafts, as they were not only very important but also sometimes humorous at the same time.

Dear Dr. Kevin Damman, before I came to Groningen, I had already read many of your papers, and I was always impressed with your articles. I was not sure if I could get the chance to work with you, so I was happy when you became one of my supervisors. Your comments, advice, and criticism are always helpful and accurate, and I have learned a lot from you. It was also a great opportunity to be a participant of the second Postgraduate Course in Heart Failure from 2016 until 2017. I look forward to getting an opportunity to collaborate with you in heart failure clinical research in the future. Please keep in touch.

Dear Jozine, thank you for helping me to begin my research at the UMCG. You taught me how things work in the UMCG and supervised many projects of mine. You have been very kind, cooperative, and provided me with the advice necessary to advance my Ph.D. project. I look forward to seeing you at scientific meetings and collaborating in the near future.

Dear Biniyam, when I was stuck on data analysis and statistics, the discussions with you helped me very much. I have learned many more things from those discussions, which have also improved my logical thinking skills. I have fond memories of flying to Florence with you to attend the HFSA meeting, and I really enjoyed having coffee with you.

Dear Niels and Haye, thank you for helping me access the PREVEND dataset and understand its

structure. It ended up as being a large part of Chapter 2 of my thesis.

Dear Alma, Audrey, Danielle, and Carla, you have all helped me from the beginning, especially when I was preparing many documents so that I could study at UMCG, and during my time over there. I want to thank you very much for all your support and help.

In addition to all Ph.D. colleagues, I would like to thank Licette, Eline, Koen, Jasper, Bastiaan, Minke, Ruben, Mohsin, Yanick, Lawien, and Niek. I really enjoyed having coffee with you all in the pantry.

Dear Tom and Abdullah, thank you for everything you did for my defense, including being my paranymphs. I was very happy to be working with you both in the same room at UMCG. Talking about anything with you both was always fun, and I was always glad that you are both interested in Japan and Japanese culture. Tom, you even actually visited Japan! I hope that we can see each other in Japan someday and I am really looking forward to your visit.

Dear Juan Diego, Olivia, Brian, Corinna, and their lovely kids (Laura, Marcos, Alina, and Johanna), thank you for becoming our best friends in the Netherlands. All of you meant a lot to us and helped us in many ways. We enjoyed doing many things on the weekend and during our vacations. During regular weekends, we had a great cultural exchange through food (and some beer and wine!), which we really loved. We already miss you very much, and hope all of us can have a reunion somewhere in the world in the near future!

Dear mom and dad, I have always felt your love and support since my childhood. When my family and I were in the Netherlands, we missed each other very much even though we Skyped almost every weekend. The experience in this country undoubtedly changed our lives, and we had a special time here. We could not do this without your help, and we always appreciate your support.

Dear Rika and Naho, it is very hard for me to express in words how much you have supported me and how much you both mean to me. It must have been difficult to start living in a different culture with different people. However, you were both amazing and settled in this new environment and encouraged me very much. I hope you have enjoyed living in this beautiful country and feel that we made great memories there. Although we have begun a new life in Tokyo, what we experienced in the Netherlands is always in our hearts, and I believe it strengthened our family bond. Once again, thank you for choosing to come with me to the Netherlands; I could not have successfully finished my Ph.D. without you.

About the author

Yuya Matsue was born on January 23, 1980, in Kagoshima, Japan. After graduating from high school (Toho University High School, Chiba, Japan), he began studying medicine at Kagoshima University School of Medicine, Kagoshima, Japan. He successfully graduated in 2005 and began a two-year internal medicine residency at Kameda Medical Center, Chiba, Japan. Afterward, he began his career as a cardiology fellow at Kameda Medical Center, Chiba, Japan, which took three years to complete. He was promoted to the position of staff physician after completing the cardiology fellowship and became interested in the medicine of heart failures. In the first five years of being a staff physician at the hospital, he served mainly as a heart failure specialist and became more interested in heart failure clinical research. In 2014, this brought him to the University Medical Center Groningen, where he joined a research team and was a Ph.D. student supervised by Prof. Dr. Adriaan A. Voors. In 2018, he returned to Japan and began working as an associate professor in the Department of Cardiology, Juntendo University School of Medicine, Tokyo, Japan.

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